



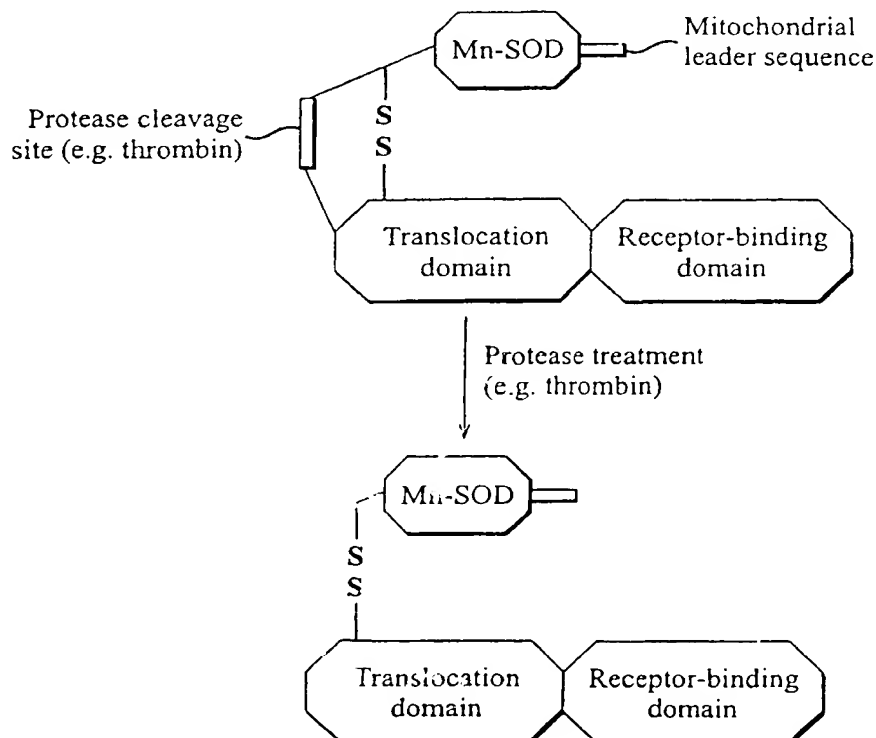
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>7</sup> : C12N 15/53, 15/62, 9/02, A61K 38/44, 42/00, C07K 14/33, A61K 39/08</p>	<p>A1</p>	<p>(11) International Publication Number: <b>WO 00/28041</b></p> <p>(43) International Publication Date: 18 May 2000 (18.05.00)</p>
<p>(21) International Application Number: PCT/GB99/03699</p> <p>(22) International Filing Date: 5 November 1999 (05.11.99)</p> <p>(30) Priority Data: 9824282.9 5 November 1998 (05.11.98) GB</p> <p>(71) Applicant (for all designated States except US): MICROBIOLOGICAL RESEARCH AUTHORITY [GB/GB]; CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): SHONE, Clifford, Charles [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB). SUTTON, John, Mark [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB). HALLIS, Bassam [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB). SILMAN, Nigel [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB).</p> <p>(74) Agents: SCHLICH, George, William et al.; Mathys &amp; Squire, 100 Gray's Inn Road, London WC1X 8AL (GB).</p>	<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TC).</p> <p><b>Published</b></p> <p><i>With international search report.</i></p> <p><i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	

(54) Title: DELIVERY OF SUPEROXIDE DISMUTASE TO NEURONAL CELLS

(57) Abstract

A composition for delivery of superoxide dismutase to neuronal cells comprise a superoxide dismutase linked by a linker to a neuronal cell targeting component, which component comprises a first domain that binds to a neuronal cell and a second domain that translocates the superoxide dismutase into the neuronal cell. After translocation, the linker is cleaved to release superoxide dismutase from the neuronal cell targeting domain. Also described is use of the composition for treatment of oxidative damage to neuronal cells and further targeting of the composition using human mitochondrial leader sequences. A hybrid polypeptide is described that contains a bacterial superoxide dismutase plus a sequence that targets a human mitochondria.



*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## DELIVERY OF SUPEROXIDE DISMUTASE TO NEURONAL CELLS

The present invention relates to compositions and methods for delivery of superoxide dismutase (SOD) to neuronal cells, and in particular for delivery of SOD to mitochondria in those cells. The present invention also provides methods of making the constructs.

A number of nervous system disorders would benefit considerably from rapid intervention with several therapeutic agents. Examples of such disorders are global, focal or spinal cord ischaemia induced by stroke or injury. Neurones injured by trauma or ischaemia produce elevated levels of transmitter substances which result in high levels of reactive oxygen species. These reactive oxygen species, in high concentrations are toxic to both the neurones and the surrounding cells which potentiates and amplifies the damage process. Rapid therapeutic intervention with agents to reduce oxidative stress in cases of neuronal injury caused by stroke or trauma could therefore significantly limit this secondary damage process.

One such potential therapeutic agent is superoxide dismutase which neutralises the harmful effects of the superoxide radicals by converting them to hydrogen peroxide and oxygen.

Francis and co-workers demonstrated that post-ischaemic infusion of Copper/Zinc superoxide dismutase (Cu/Zn-SOD) reduces cerebral infarction following ischaemia/reperfusion in rats (Experimental Neurology (1997) 146, 435-443) through the reduction of damaging free-radical oxygen. Lim et al. have shown that administration of Cu/Zn-SOD attenuates the level of reperfusion injury following spinal cord ischaemia in dogs (Ann. Thorac. Surg. (1986) 42, 282-286). Cuevas et al. have similarly demonstrated protective effects of SOD, both on neurological recovery and spinal infarction, in ischaemic reperfusion injury of the rabbit spinal cord (Acta Anat. (1990) 137, 303-310). A major problem in the use of such therapies is the maintenance of useful concentration of the active agent at the site

- 2 -

of trauma. Enzymes such as Cu/Zn-SOD are rapidly cleared from the systemic circulation; in the case of the latter enzyme the  $t_{1/2}$  in rat is 4-8 minutes. A number of strategies have been employed to overcome these difficulties. Matsumiya et al. (Stroke (1991) 22, 1193-1200) conjugated  
5 Cu/Zn-SOD to polyethylene glycol to increase its half-life in the blood. Francis and co-workers (Experimental Neurology (1997) 146, 435-443) describe the neuronal delivery of Cu/Zn-SOD by fusion of the enzyme to the binding domain of tetanus toxin. None of these strategies, however, are particularly efficient at delivering the enzyme to the intracellular  
10 neuronal compartments where the enzyme can be effective.

In most eukaryotic species, two intracellular forms of superoxide dismutase exist: the Cu/Zn-SOD which is located within the cytoplasmic and nuclear compartments and manganese superoxide dismutase (Mn-SOD) which is  
15 located within the mitochondrial matrix. Human Mn-SOD is a tetrameric enzyme and is larger than the dimeric Cu/Zn-SOD. Several studies have shown that decreased Mn-SOD may be associated with one or more chronic diseases such as ovarian cancer (Nishida *et al.* (1995) Oncology Reports, 2, 643-646) and diabetes (L'Abbe *et al.*, (1994) Proc Soc Exp Biol  
20 Med, 207, 206-274). In addition, mice in which the Mn-SOD gene has been knocked out exhibit several novel pathogenic phenotypes including severe anaemia, degeneration of neurones in the basal ganglia and brainstem, and progressive motor disturbances characterised by weakness and rapid fatigue (Lebovitz *et al.*, (1996) Proc Natl Acad Sci USA, 93,  
25 9782-9787). In addition these mice showed extensive damage to the neuronal mitochondria. Overexpression of Mn-SOD in cell lines and transgenic mice showed that damage and apoptosis of neurones under oxidative stress was markedly reduced (Keller *et al.*, (1998) Journal of Neuroscience, 18, 687-697). Mitochondrial damage was also reduced.  
30 These data showed that superoxide accumulation and subsequent mitochondrial damage play key roles in neuronal death induced by trauma both *in vitro* and *in vivo*. Delivery of agents which reduce the level of

- 3 -

oxidative stress to neuronal cells may therefore reduce neuronal cell death and afford considerable therapeutic benefits.

5 Mn-SODs of bacterial origin such as that from *Bacillus stearothermophilus* or *B. caldotenax* consist of two subunits and are smaller than the human isoform which is a tetramer. *B. stearothermophilus* and *B. caldotenax* Mn-SOD also have considerably lower immunogenicity than the human isoform which is an advantage for continued therapeutic use. As enzymes for therapeutic applications, however, they suffer from similar drawbacks to  
10 other SODs in that very little of the administered enzyme is retained within the tissues where it would be therapeutically beneficial.

The botulinum neurotoxins are a family of seven structurally similar, yet antigenically different, protein toxins whose primary site of action is the  
15 neuromuscular junction where they block the release of the transmitter acetylcholine. The action of these toxins on the peripheral nervous system of man and animals results in the syndrome botulism, which is characterised by widespread flaccid muscular paralysis (Shone (1986) in 'Natural Toxicants in Foods', Editor D. Watson, Ellis Harwood, UK). Each  
20 of the botulinum neurotoxins consist of two disulphide-linked subunits; a 100 kDa heavy subunit which plays a role in the initial binding and internalisation of the neurotoxin into the nerve ending (Dolly et. al. (1984) Nature, 307, 457-460) and a 50 kDa light subunit which acts intracellularly to block the exocytosis process (McInnes and Dolly (1990) Febs Lett., 261, 323-326; de Paiva and Dolly (1990) Febs Lett., 277, 171-174). Thus it is  
25 the heavy chains of the botulinum neurotoxins that impart their remarkable neuronal specificity.

30 Tetanus toxin is structurally very similar to botulinum neurotoxins but its primary site of action is the central nervous system where it blocks the release of inhibitory neurotransmitters from central synapses (Renshaw cells). As described for the botulinum toxins above, it is domains within

- 4 -

the heavy chain of tetanus toxin that bind to receptors on neuronal cells.

The binding and internalisation (translocation) functions of the clostridial neurotoxin (tetanus and botulinum) heavy chains can be assigned to at least two domains within their structures. The initial binding step is energy-independent and appears to be mediated by one or more domains within the H<sub>C</sub> fragment of the neurotoxin (C-terminal fragment of approximately 50kDa) (Shone *et al.* (1985), Eur. J. Biochem., 151, 75-82) while the translocation step is energy-dependent and appears to be mediated by one or more domains within the H<sub>N</sub> fragment of the neurotoxin (N-terminal fragment of approximately 50kDa).

Isolated heavy chains are non-toxic compared to the native neurotoxins and yet retain the high affinity binding for neuronal cells. Tetanus and the botulinum neurotoxins from most of the seven serotypes, together with their derived heavy chains, have been shown to bind a wide variety of neuronal cell types with high affinities in the nM range (e.g botulinum type B neurotoxin; Evans *et al.* (1986) Eur. J. Biochem. 154, 409-416).

Another key characteristic of the binding of these neurotoxins is that tetanus, botulinum A, B, C<sub>1</sub>, D, E and F neurotoxins all appear to recognise distinct receptor populations, and collectively the clostridial neurotoxin heavy chains provide high affinity binding ligands that recognise a whole family of receptors that are specific to neuronal cells.

However, whilst it is known to provide a fusion of a SOD with a neurotoxin heavy chain, this fusion has been found to be ineffective for delivery of SOD to neuronal cells and inactive in *in vitro* assays for potential therapeutic activity.

It is an object of the invention to provide compositions and methods for delivery of SOD to neuronal cells. A further object is to provide

- 5 -

compositions and methods for treatment or at least amelioration of conditions such as ischemic stroke.

Accordingly, the present invention provides a construct for delivering  
5 superoxide dismutase (SOD) to neuronal cells. In one aspect of the invention the construct consists of a SOD which has been combined with various functional protein domains to effect efficient targeting to the mitochondria within neuronal cells. The construct of specific embodiments of the invention, described in further detail below, contains the following  
10 elements:-

- a SOD which contains a leader sequence for targeting SOD to the mitochondria;
- a dimeric SOD which has low immunogenicity and high stability;
- a SOD which is linked to a domain that effects translocation across  
15 lipid membranes;
- a linkage between the SOD and the translocation domain that is cleaved within the neuronal cytosol; and
- a domain which selectively targets the construct to neuronal cells.

20 A first aspect of the invention thus provides a composition for delivery of superoxide dismutase (SOD) to neuronal cells, comprising:-

SOD; linked by a cleavable linker to  
a neuronal cell targeting component, comprising a first domain that  
binds to a neuronal cell and a second domain that translocates the  
25 SOD of the composition into the neuronal cell.

The linker is cleavable and thus, in use, after translocation of the SOD into the cell, the linker is cleaved to release SOD from the neuronal cell targeting domain. A suitable linker is a disulphide bridge between cysteine  
30 residues, one residue on the SOD and one residue on the neuronal cell targeting component, for example on the second domain. Another example of a linker is a site for a protease found in neuronal cells. In this way, the

- 6 -

linker is such that it is cleaved within the cell, separating the SOD from the other translocated portions of the composition.

5 By SOD is meant a sequence that has superoxide dismutase activity, and may also comprise a further sequence or sequences conferring additional properties on that portion of the constructs. For example, the SOD optionally also includes a sequence targeting the SOD to mitochondria in a neuronal cell.

10 The SOD may be a hybrid of Mn-SOD and a sequence targeting the hybrid to mitochondria. The SOD may be of bacterial or human origin, or a derivative thereof, and may be comprised of sequences from more than one origin, provided that it has superoxide dismutase activity.

15 The first domain may suitably be selected from (a) neuronal cell binding domains of clostridial toxins; and (b) fragments, variants and derivatives of the domains in (a) that substantially retain the neuronal cell binding activity of the domains of (a). The second domain is suitably selected from (a) domains of clostridial neurotoxins that translocate polypeptide sequences  
20 into cells, and (b) fragments, variants and derivatives of the domains of (a) that substantially retain the translocating activity of the domains of (a).

In an embodiment of the invention a construct comprises SOD linked by a disulphide bridge to a neuronal cell targetting component comprising a first  
25 domain that binds to a neuronal cell and a second domain that translocates the SOD into the neuronal cell. This construct is made recombinantly as a single polypeptide having a cysteine residue on the SOD which forms a disulphide bridge with a cysteine residue on the second domain. The SOD is covalently linked, initially, to the second domain. Following expression  
30 of this single polypeptide SOD is cleaved from the second domain leaving the SOD linked only by the disulphide bridge to the rest of the construct.



- 7 -

A second aspect of the invention provides a pharmaceutical composition for treatment of oxidative damage to neuronal cells comprising a composition according to the invention with a pharmaceutically acceptable carrier. This composition may be used to deliver SOD to a neuronal cell,  
5 for example by administration of the composition by injection.

A third aspect of the invention provides a method of preparing a composition according to the invention, comprising chemically linking SOD, linker and neuronal cell targeting components. The SOD preferably is free  
10 of cysteine residues and the method preferably comprises treating the SOD with a cross-linker which will form a disulphide bridge with a cysteine residue on the neuronal cell targeting domain.

In a further embodiment of the third aspect of the invention, there is  
15 provided a method of making a composition according to the invention comprising expressing a DNA that codes for a polypeptide having SOD, a linker, a neuronal cell targeting component. The polypeptide may further comprise a purification sequence and the method may further comprise purifying the polypeptide using this sequence and then cleaving the  
20 polypeptide to remove the purification sequence to leave SOD, the linker and the neuronal cell targeting component.

By virtue of the combination of properties defined above, constructs of the invention are surprisingly efficient at transporting SOD to the mitochondria  
25 within neuronal cells. The ability of the superoxide dismutase to be translocated into the cytosol by virtue of the 'translocation domain' within the construct and the cleavage of the enzyme from the latter domain within the cell is key to this targeting efficiency. As such the construct of the invention has considerable therapeutic value in treating neuronal diseases  
30 which results from oxidative stress and has several advantages over previously described SOD formulations. Mitochondria within cells containing high levels of superoxide radicals are particularly sensitive to

- 8 -

damage and the ability of the construct of the invention to target the SOD to these organelles offers considerable advantage in that the enzyme can act to remove the superoxide radicals where it is most required.

5 The construct of the invention may be used clinically in a variety of neuronal diseases which are caused or augmented by oxidative stress. Such conditions include ischaemic stroke, Parkinson's disease, Huntington's disease and motor neurone diseases. In the case of ischaemia/reperfusion injury caused by stroke or trauma, delivery of the construct of the invention  
10 to neurones of the hippocampus may afford considerable therapeutic benefits by reducing neuronal damage and death. Other neuronal diseases where the underlying cause is oxidative stress would also benefit from the therapeutic effects of the construct of the invention.

15 In a preferred aspect of the invention, the SOD is a dimeric, manganese superoxide dismutase (Mn-SOD) which is of bacterial origin and has low immunogenicity and high stability.

20 The use of a bacterial Mn-SOD in constructs has a number of advantages compared to the use of the human Mn-SOD isoform:-

- the low immunogenicity of the bacterial Mn-SOD is advantageous where repeated administration of the construct is required, in which cases the induction of adverse host immune responses is reduced;  
25 and

- the smaller size of constructs based on the dimeric bacterial Mn-SOD compared to human Mn-SOD (which is a tetramer) both reduces the likelihood of adverse immune responses and increases  
30 the rate of diffusion of the construct to its target tissue.

In exercise of an example of the invention, a bacterial Mn-SOD of low

- 9 -

immunogenicity is derived from either *B. stearothermophilus* (sequence as reported by Brock and Walker (1980) Biochemistry, 19, 2873-2882) or *B. caldotenax* (gene and amino acid sequence as defined by Chambers *et al.*, (1992) FEMS Microbiology Letters, 91, 277-284) to which a mitochondrial leader sequence has been fused to the N-terminus of the protein by recombinant technology. This Mn-SOD-leader hybrid is linked by a disulphide bridge to a translocation domain derived from a bacterial protein toxin, such as botulinum neurotoxin. The translocation domain, in turn is fused to a receptor binding domain derived from a clostridial neurotoxin (botulinum or tetanus). The construct is produced initially as a single polypeptide by recombinant technology and subsequently converted to the construct of the invention by selective cleavage with a proteolytic enzyme. To produce the construct of the invention, a loop motif containing a unique protease site (e.g amino acid sequences specifically cleaved by proteases such as factor Xa, enterokinase, thrombin) and a cysteine residue is introduced between the C-terminus of the Mn-SOD and the N-terminus of the translocation domain such that a disulphide bridge is formed between the Mn-SOD and the translocation domain. Subsequent cleavage of the protease site generates the active construct. The final construct, when analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis in the presence of a reducing agent (e.g. dithiothreitol), dissociates into two major bands, one corresponding to the superoxide dismutase enzyme and a second corresponding to a polypeptide which contains the neuronal binding and translocation domains. In the absence of a reducing agent this dissociation does not occur and the complex is observed as a single major band on the gels.

Modification of Mn-SOD from *B. stearothermophilus* by addition of a mitochondrial targeting sequence offers several advantages over the use of human Mn-SOD which contains its own mitochondrial leader sequence. Firstly, the *B. stearothermophilus* Mn-SOD has a high thermal stability and low immunogenicity which allows administration of several doses of the

- 10 -

enzyme without provoking an immune response from the host that would reduces its efficacy. Secondly, the *B. stearothermophilus* Mn-SOD is a small dimeric enzyme unlike the human Mn-SOD which is a tetramer. Recombinant constructs containing the latter enzyme would therefore have to be considerably larger and more complex in their structure.

In an embodiment of the invention, a DNA encoding a construct of the invention is made up by fusion of following DNA fragments commencing at the 5' end of the gene:-

an oligonucleotide encoding a modified human mitochondrial leader sequence (amino acid sequence: MLSRAVCGTSRQLAPALGYLGSRQ (SEQ ID NO:10) or MLSRAVSGTSRQLAPALGYLGSRQ (SEQ ID NO:11));

an oligonucleotide encoding Mn-SOD from *B. stearothermophilus* (coding for the amino acid sequence as defined in Brock and Walker (1980) Biochemistry, 19, 2873-2882);

an oligonucleotide encoding a linker peptide which contains the thrombin protease cleavage site and a cysteine residue for disulphide bridge formation (peptide sequence: CGLVPAGSGP);

an oligonucleotide encoding a translocation domain derived from a botulinum neurotoxin (e.g. a DNA fragment coding for amino acid residues 449-871 of botulinum type A neurotoxin, or a DNA fragment coding for amino acid residues 441-858 of botulinum type B neurotoxin, or a DNA fragment coding for amino acid residues 440-864 of botulinum type F neurotoxin); and

an oligonucleotide encoding the receptor binding domain of a botulinum neurotoxin or tetanus neurotoxin (e.g. a DNA fragment

- 11 -

coding for amino acid residues 872-1296 of botulinum type A neurotoxin, or a DNA fragment coding for amino acid residues 859-1291 of botulinum type B neurotoxin, or a DNA fragment coding for amino acid residues 865-1278 of botulinum type F neurotoxin, or a DNA fragment coding for amino acid residues 880-1315 of tetanus neurotoxin).

The above DNA fragments may be obtained and constructed by standard recombinant DNA methods. Expression and purification of the assembled construct may be obtained with a variety of suitable expression hosts, e.g. *Escherichia coli*, *Bacillus subtilis*.

The translocation domain and neuronal binding domain of the construct may also be derived from combination of different clostridial neurotoxins. For example, the construct of the invention may contain a translocation domain derived from botulinum type F neurotoxin and a binding domain derived from botulinum type A neurotoxin.

A construct of the invention may be produced using protein chemistry techniques. Mn-SOD derived from *B. stearothermophilus* to which a mitochondrial leader sequence has been fused to the N-terminus of the protein by recombinant technology is modified with a heterobifunctional cross-linking reagent such as N-succinimidyl 3-[2-pyridyldithio] propionate (SPDP). The chemically modified enzyme is then combined to a cell targetting domain which contains the binding and translocation functional domains. The latter may be produced by recombinant technology or purified from the neurotoxins of *Clostridium botulinum* or *Clostridium tetani* by established methods. Chemical coupling of the SPDP-treated Mn-SOD may be accomplished using a free cysteine residue on the polypeptide containing the binding and translocation domains to give a construct of the invention.

Constructs of the invention may be introduced into either neuronal or non-neuronal tissue using methods known in the art. By subsequent specific binding to neuronal cell tissue, the targeted construct will exert its therapeutic effects. Alternatively, the construct may be injected near a site requiring therapeutic intervention, e.g. intrathecal or intracranial injection close to a site of trauma or disease.

The construct of the invention may also be administered with other agents which enhance its delivery to its target tissue. An example of such an agent is one which assists the passage of the construct of the invention through the blood-brain barrier to the central nervous system. The construct of the invention may also be administered in formulations with other therapeutic agents or drugs.

The dosage required for the construct of the invention will depend upon the application and could vary between 1 $\mu$ g/kg to 100mg/kg of body weight. The construct of the invention may be produced as a suspension, emulsion, solution or as a freeze dried powder depending on the application and properties of the release vehicle and its therapeutic contents. The construct of the invention may be resuspended or diluted in a variety of pharmaceutically acceptable liquids depending on the application.

“Clostridial neurotoxin” means a neurotoxin corresponding to tetanus neurotoxin or one of the seven botulinum neurotoxin serotypes (type A, B, C<sub>1</sub>, D, E, F or G).

"Bind" in relation to the clostridial binding fragments, means the interaction between the clostridial fragment and one or more cell surface receptors or markers which results in localisation of the binding fragment or construct in the vicinity of the cell.

"Binding domain" of botulinum or tetanus neurotoxins means a domain of

- 13 -

the toxin which retains the property of being able to bind the receptors on neuronal cells in a similar manner to the intact neurotoxin and encompasses native domains and fragments, variants and derivatives that retain this binding function. This property of the binding domain can be assessed in competitive binding assays. In such assays, radiolabelled neurotoxin (e.g. botulinum type A neurotoxin) is contacted with neuronal cells in the presence of various concentrations of non-radiolabelled fragment representing the 'binding domain' of the neurotoxin. The ligand mixture is incubated with the cells, at low temperature (0-3°C) to prevent ligand internalisation, during which competition between the radiolabelled neurotoxin and non-labelled 'binding domain' fragment may occur. In such assays when the unlabelled ligand used is binding domain of botulinum type A neurotoxin (residues 872-1296), the radiolabelled botulinum type A neurotoxin will be displaced from the neuronal cell receptors as the concentration of its non-labelled 'binding domain' is increased. The competition curve obtained in this case will therefore be representative of the behaviour of a 'binding domain' fragment being able to bind the receptors on neuronal cells in a similar manner to the intact neurotoxin. This property of the binding domain may be used to identify other suitable protein domains which have the desired binding properties. Examples of binding domains derived from clostridial neurotoxins are as follows:-

Botulinum type A neurotoxin	- amino acid residues (872 - 1296)
Botulinum type B neurotoxin	- amino acid residues (859 - 1291)
Botulinum type C neurotoxin	- amino acid residues (867 - 1291)
Botulinum type D neurotoxin	- amino acid residues (863 - 1276)
Botulinum type E neurotoxin	- amino acid residues (846 - 1252)
Botulinum type F neurotoxin	- amino acid residues (865 - 1278)
Botulinum type G neurotoxin	- amino acid residues (864 - 1297)
Tetanus neurotoxin	- amino acid residues (880 - 1315)

"Translocation domain" means a domain or fragment of a protein which effects transport of itself and/or other proteins and substances across a

- 14 -

membrane or lipid bilayer and encompasses native domains and fragments, variants and derivatives that retain this binding function. The latter membrane may be that of an endosome where translocation will occur during the process of receptor-mediated endocytosis. Translocation domains can frequently be identified by the property of being able to form measurable pores in lipid membranes at low pH (Shone *et al.* (1987) Eur J. Biochem. 167, 175-180). The latter property of translocation domains may thus be used to identify other protein domains which could function as the translocation domain within the construct of the invention. Examples of translocation domains derived from bacterial neurotoxins are as follows:-

Botulinum type A neurotoxin	- amino acid residues (449 - 871)
Botulinum type B neurotoxin	- amino acid residues (441 - 858)
Botulinum type C neurotoxin	- amino acid residues (442 - 866)
Botulinum type D neurotoxin	- amino acid residues (446 - 862)
Botulinum type E neurotoxin	- amino acid residues (423 - 845)
Botulinum type F neurotoxin	- amino acid residues (440 - 864)
Botulinum type G neurotoxin	- amino acid residues (442 - 863)
Tetanus neurotoxin	- amino acid residues (458 - 879)

"Translocation" in relation to translocation domain, means the internalisation events which occur after modified clostridial binding fragments bind to the cell surface. These events lead to the transport of substances into the cytosol of neuronal cells.

"Unique protease site" means a protease site incorporated into the construct such that the molecule may be proteolysed at pre-determined sites by a selected protease. The specificity of these proteases is such that cleavage to other parts of the construct does not occur. Examples of unique protease sites are the amino acid sequences cleaved by proteases such as: thrombin, factor Xa, enterokinase.

A fourth aspect of the invention provides a composition for delivery of a



- 15 -

therapeutic agent to neuronal cells, comprising:-

the therapeutic agent; linked by a cleavable linker to  
a neuronal cell targeting component, comprising a first domain that  
binds to a neuronal cell and a second domain that translocates the  
therapeutic agent of the composition into the neuronal cell.

Thus, in use, after translocation of the therapeutic agent into the cell, the  
linker is cleaved to release the therapeutic agent from the neuronal cell  
targeting domain. Other optional and preferred embodiments of the fourth  
aspect of the invention are as for the first-third aspects of the invention.

A fifth aspect of the invention provides a polypeptide comprising a  
bacterial SOD, or derivative thereof, and a sequence for targeting the  
polypeptide to a mitochondria, such as a human mitochondria. The  
polypeptide may be chemically obtained by synthesis of otherwise or may  
be a fusion protein, obtained for example by expression of a nucleotide  
coding for the polypeptide.

The invention hence also provides, in a sixth aspect, a nucleotide encoding  
the polypeptide of the fifth aspect and in a seventh aspect a vector  
comprising the nucleotide of the sixth aspect. Also provided in an eighth  
aspect is a method of making a polypeptide according to the fifth aspect  
comprising expressing the nucleotide sequence of the sixth aspect. In a  
ninth aspect is provided a cell comprising the nucleotide sequence of the  
sixth aspect or the vector of the seventh.

There now follows description of specific embodiments of the invention  
illustrated by drawings in which:-

Fig. 1 shows schematic examples of novel Mn-SODs derived from  
*B. stearothermophilus* and *B. caldotenax*. Two examples of mitochondrial  
leader sequences are shown. In one example, a cysteine residue at position

- 16 -

7 has been mutated to a serine residue. This change enables the production of the construct of the invention without the formation of disulphide bridges in undesirable positions;

5           Figure 2 shows schematic examples of novel Mn-SOD fusion proteins showing the use of peptides and proteins to facilitate purification of the enzyme from the production strain. Various protein and peptide tags (such as histidine-6, S-peptide, maltose-binding protein, calmodulin-binding protein) may be fused to the Mn-SOD to allow rapid purification by affinity  
10 chromatography methods. Unique protease sites are incorporated between the purification tag and the Mn-SOD to enable removal of the tag after purification. Protein and peptide tags may be removed by treatment of the fusion protein with the relevant specific protease (e.g. factor Xa, thrombin, enterokinase);

15           Fig. 3 shows a recombinant Mn-SOD construct of the invention. From the N-terminus of the protein, the construct consists of the following components:- (1) a mitochondrial leader (targeting) sequence, (2) a Mn-superoxide dismutase, (3) a loop which contains a unique protease site and  
20 which allows disulphide bridge formation, (4) a translocation domain, (5) a neuronal targeting domain. The construct is produced as a single polypeptide; subsequent cleavage with a protease specific for the 'unique protease site' contained within the loop region generates the di-chain construct. Purification tags could added to the constructs as exemplified  
25 in Figure 3;

30           Fig. 4 shows the production of a Mn-SOD construct by chemical methods. The method uses a recombinant Mn-SOD, purified as described in Example 1 and coupled to a polypeptide containing the translocation and binding domains as described in Example 4; and

Fig. 5 shows the results of an example to demonstrate the protective

- 17 -

effects of a construct of the invention on NG108 cells subjected to oxidative stress by the addition of 50 $\mu$ M duroquinone for four hours.

The application is also accompanied by a sequence listing in which:-

5

SEQ ID NO: 1 shows the amino acid sequence of Mn-SOD from *B. caldotenax*;

10

SEQ ID NO: 2 shows the amino acid sequence of Mn-SOD from *B. stearothermophilus*;

15

SEQ ID NO: 3 shows the amino acid sequence of a construct of the invention comprising Mn-SOD from *B. stearothermophilus*, a linker that can be cleaved by thrombin, and a heavy chain derived from botulinum neurotoxin serotype A;

20

SEQ ID NO: 4 shows the amino acid sequence of a construct of the invention comprising Mn-SOD from *B. stearothermophilus*, a linker that can be cleaved by thrombin, and a heavy chain derived from botulinum neurotoxin serotype B;

25

SEQ ID NO: 5 shows the amino acid sequence of a construct of the invention comprising Mn-SOD from *B. stearothermophilus*, a linker that can be cleaved by thrombin, and a heavy chain derived from botulinum neurotoxin serotype F;

30

SEQ ID NO: 6 shows the amino acid sequence of a construct of the invention comprising a mitochondrial leader sequence from human Mn-SOD, Mn-SOD from *B. stearothermophilus*, a linker that can be cleaved by thrombin, and a heavy chain derived from botulinum neurotoxin serotype A;

- 18 -

SEQ ID NO: 7 shows the amino acid sequence of a construct of the invention comprising a mitochondrial leader sequence from human Mn-SOD, Mn-SOD from *B. stearothermophilus*, a linker that can be cleaved by thrombin, and a heavy chain derived from botulinum neurotoxin serotype B;

SEQ ID NO: 8 shows the amino acid sequence of a construct of the invention comprising a mitochondrial leader sequence from human Mn-SOD, Mn-SOD from *B. stearothermophilus*, a linker that can be cleaved by thrombin, and a heavy chain derived from botulinum neurotoxin serotype F; and

SEQ ID NO: 9 shows the amino acid sequence for a polypeptide comprising a mitochondrial leader sequence from human Mn-SOD and Mn-SOD from *B. stearothermophilus*;

SEQ ID NO: 10 shows the amino acid sequence of a modified human mitochondrial leader sequence; and

SEQ ID NO: 11 shows an amino acid sequence of a modified human mitochondrial leader sequence.

#### Example 1.

#### **Production and purification of novel *B. stearothermophilus* Mn-SOD containing a mitochondrial leader sequence.**

Standard molecular biology protocols were used for all genetic manipulations (eg. Sambrook *et al.* 1989, Molecular Cloning a Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). A synthetic gene encoding the mitochondrial targeting sequence (amino acids 1-27) of the human Mn-SOD gene was cloned as

- 19 -

an *NdeI* - *Bam* HI fragment into an expression vector so that the transcriptional start corresponds to the ATG codon within the *NdeI* site. The Mn-SOD gene from *B. stearrowthermophilus* or *B. caldotenax* was amplified using PCR to give a *Bgl*III site corresponding to the leucine amino acid at position 5 at the 5' end, and a *Bam*HI site outside the stop codon at the 3' end. This *Bs*III-*Bam*HI fragment was cloned into the expression vector carrying the mitochondrial targeting sequence (digested *Bam*HI) to generate "in-frame" gene fusions. In addition to the wild type mitochondrial targeting sequence, a variant was constructed in which the cysteine at position 7 was changed to serine

The recombinant Mn-SOD expressed in pET28a were produced with amino-terminal histidine (6 His) and T7 peptide tags allowing proteins to be purified by affinity chromatography on either a  $\text{Ni}^{2+}$  charged column or an anti-T7 immunoaffinity column (Smith *et al.* 1988, *Journal of Biological Chemistry*, 263: 7211-7215). Incorporation of a factor Xa protease cleavage between the peptide tag and the expressed Mn-SOD allowed this to be removed after purification. Briefly, cultures of *E.coli* BL21 (DE3) pET28a-Mn-SOD were grown in Terrific broth-kanamycin ( $30 \mu\text{gml}^{-1}$ ) to an  $\text{OD}_{600 \text{ nm}}$  of 2.0, and protein expression was induced by the addition of  $500 \mu\text{M}$  IPTG for approximately 2 h. Cells were lysed by freeze/thaw followed by sonication, lysates cleared by centrifugation and supernatants loaded onto an anion exchange column (MonoQ™ column on a Fast Protein Liquid Chromatography system; Pharmacia Biotech, Uppsala, Sweden). Eluted recombinant Mn-SOD was then desalted and further purified by affinity chromatography on a chelating sepharose column charged with  $\text{Ni}^{2+}$  (Pharmacia Biotech, Uppsala, Sweden). After loading proteins onto the column and subsequent washing, the purified Mn-SOD was eluted with imidazole. All buffers used were as specified by the manufacturer

A 'maltose binding protein' purification tag was also employed for the purification of some batches of Mn-SOD. The use of this system is

- 20 -

described in detail in New England Biolabs Instruction Manual "Protein Fusion and Purification System" (ver 3.02).

5 Other tags and protease cleavage site may also be incorporated into the sequence to facilitate purification of Mn-SOD as exemplified in Figure 2.

For purification of a novel *B. stearothermophilus* Mn-SOD which was not conjugated to protein purification tag, the following procedure was used. After harvesting, cells were broken by high pressure homogenisation crude  
10 extracts were clarified by centrifugation and batch purified on DE-23 cellulose. The fraction eluted with 0.4M NaCl contained the Mn-SOD. This fraction was then further purified by various chromatographic media using the following sequence:-

15 DEAE-Sepharose ion exchange chromatography at pH 8.0; elution of the Mn-SOD with a NaCl gradient;

hydroxylapatite chromatography at pH 6.8; elution of Mn-SOD with a phosphate gradient at pH 6.8;

20 ion exchange chromatography on Q-Sepharose at pH 7.5; elution with a NaCl gradient; and

gel filtration on Sephacryl S-200.

25 The purified Mn-SOD may be dialysed against Hepes buffer (0.1M, pH7.4) containing 0.15M NaCl and stored at -80°C.

### Example 2.

30 Preparation and purification of a recombinant Mn-SOD construct of the invention.

- 21 -

Standard molecular biology protocols were used for all genetic manipulations (*eg.* Sambrook *et al.* 1989, Molecular Cloning a Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). Various DNA fragments of the construct were generated using Recursive PCR reactions (Prodromou & Pearl 1992, *Protein Engineering*, 5: 827-829) using self-priming oligonucleotides containing the desired sequence. For the expression of clostridial neurotoxin fragments the codon bias and GC/AT base ratio was adjusted for ease of expression in *E. coli*. Fragments were cloned sequentially into pLitmus 38 (New England Biolabs, Inc., Beverly, MA) to assemble the entire gene. Constructs for expression were sub-cloned into pET28b (Novagen Inc., Madison, WI) replacing the *EcoR1-HindIII* fragment. The ligation reactions were transformed into *E. coli* DH5 $\alpha$  (Life Technologies Inc., Gaithersburg, MD). Plasmid DNA was amplified, purified and screened for the presence of the appropriate sequence (Ausubel *et al.* 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Gene constructions confirmed as possessing the correct sequences were then transformed into the expression host *E. coli* BL21 (DE3) (Studier & Moffatt 1986, *Journal of Molecular Biology*, 189: 113-130).

The recombinant constructs expressed in pET28 were produced with amino-terminal histidine (6 His) and T7 peptide tags allowing proteins to be purified by affinity chromatography on either a Ni<sup>2+</sup> charged column or an anti-T7 immunoaffinity column (Smith *et al.* 1988, *Journal of Biological Chemistry*, 263: 7211-7215). Incorporation of a factor X protease cleavage between the peptide tags and the expressed Mn-SOD Constructs allowed these to be removed after purification. Briefly, cultures of *E. coli* BL21 (DE3) pET28-Mn-SOD Construct were grown in Terrific broth-kanamycin (30  $\mu$ gml<sup>-1</sup>) to an OD<sub>600 nm</sub> of 2.0, and protein expression was induced by the addition of 500  $\mu$ M IPTG for approximately 2 h. Cells were lysed by freeze/thaw followed by sonication, lysates cleared by centrifugation and supernatants loaded onto an anion exchange column (MonoQ™ column on

- 22 -

a Fast Protein Liquid Chromatography system; Pharmacia Biotech, Uppsala, Sweden). Eluted recombinant Mn-SOD Construct was then desalted and further purified by affinity chromatography on a chelating sepharose column charged with  $\text{Ni}^{2+}$  (Pharmacia Biotech, Uppsala, Sweden). After loading proteins onto the column and subsequent washing, the purified Construct was eluted with imidazole. All buffers used were as specified by the manufacturer.

A 'maltose binding protein' purification tag was also employed for the purification some batches of Mn-SOD Constructs. The use of this system is described in detail in New England Biolabs Instruction Manual "Protein Fusion and Purification System" (ver 3.02).

It would also be evident to anyone skilled in the art that other tags and protease cleavage site may also be incorporated into the sequence to facilitate purification as exemplified in Figure 3.

The amino sequences of several recombinant Mn-SOD constructs are shown in the sequence listing.

### Example 3.

#### Preparation of botulinum heavy chains by chemical methods.

The various serotypes of the clostridial neurotoxins may be prepared and purified from various toxigenic strains of *Clostridium botulinum* and *Clostridium tetani* by methods employing standard protein purification techniques as described previously (Shone and Tranter 1995, Current Topics in Microbiology, 194, 143-160; Springer). Samples of botulinum neurotoxin (1mg/ml) are dialysed against a buffer containing 50mM Tris-HCl pH 8.0, 1M NaCl and 2.5M urea for at least 4 hours at 4°C and then made 100mM with dithiothreitol and incubated for 16h at 22°C. The cloudy



- 23 -

solution which contains precipitated light chain is then centrifuged at 15000 x g for 2 minutes and the supernatant fluid containing the heavy chain retained and dialysed against 50mM Hepes pH 7.5 containing 0.2M NaCl and 5mM dithiothreitol for at least 4 hours at 4°C. The dialysed heavy chain is centrifuged at 15000 x g for 2 minutes and the supernatant retained and dialysed thoroughly against 50mM Hepes pH 7.5 buffer containing 0.2M NaCl and stored at -70°C. The latter procedure yields heavy chain >95% pure with a free cysteine residue which can be used for chemical coupling purposes. Biological (binding) activity of the heavy chain may be assayed as described in Example 5.

The heavy chains of the botulinum neurotoxins may also be produced by chromatography on QAE Sephadex as described by the methods in Shone and Tranter (1995) (Current Topics in Microbiology, 194, 143-160; Springer).

#### Example 4

##### Production of Mn-SOD constructs by chemical methods

*B. stearothermophilus* Mn-SOD fused to a mitochondrial leader sequence was purified as described in Example 1. The Mn-SOD was chemically modified by treatment with a 3-5 molar excess of N-succinimidyl 3-[2-pyridyldithio] propionate (SPDP) in 0.05M Hepes buffer pH 7.0 containing 0.1M NaCl for 60 min at 22°C. The excess SPDP was removed by dialysis against the same buffer at 4°C for 16h. The substituted SOD was then mixed in a 1:2.5 molar ratio with heavy chain purified from *Clostridium botulinum* type A neurotoxin purified as described in Example 3 and incubated at 4°C for 16h. During the incubation period the Mn-SOD was conjugated to the botulinum heavy chain fragment by free sulphhydryl groups (see Figure 4). After incubation, the Mn-SOD-construct was purified by gel filtration chromatography on Sephadex G200.

- 24 -

Constructs of the invention may also be formed by the above method using polypeptides containing the translocation and binding domains that have been produced by recombinant technology as outlined in Example 2.

## 5      **Example 5.**

### **Assay of the biological activity of constructs - demonstration of high affinity binding to neuronal cells.**

10      Clostridial neurotoxins may be labelled with <sup>125</sup>-iodine using chloramine-T and its binding to various cells assessed by standard methods such as described in Evans *et al.* 1986, Eur J. Biochem., 154, 409 or Wadsworth *et al.* 1990, Biochem. J. 268, 123). In these experiments the ability of Mn-SOD constructs to compete with native clostridial neurotoxins for  
15      receptors present on neuronal cells or brain synaptosomes was assessed. All binding experiments were carried out in binding buffers. For the botulinum neurotoxins this buffer consisted of: 50mM HEPES pH 7.0, 30mM NaCl, 0.25% sucrose, 0.25% bovine serum albumin. For tetanus toxin, the binding buffer was: 0.05M tris-acetate pH 6.0 containing 0.6%  
20      bovine serum albumin. In a typical binding experiment the radiolabelled clostridial neurotoxin was held at a fixed concentration of between 1-20nM. Reaction mixtures were prepared by mixing the radiolabelled toxin with various concentrations of unlabelled neurotoxin or construct. The reaction mixture were then added to neuronal cells or rat brain  
25      synaptosomes and then incubated at 0-3°C for 2hr. After this period the neuronal cells or synaptosomes were washed twice with binding ice-cold binding buffer and the amount of labelled clostridial neurotoxin bound to cells or synaptosomes was assessed by  $\gamma$ -counting. In an experiment using  
30      an Mn-SOD construct which contained the binding domain from botulinum type A neurotoxin, the construct was found to compete with <sup>125</sup>I-labelled botulinum type A neurotoxin for neuronal cell receptors in a similar manner to unlabelled native botulinum type A neurotoxin. These data showed that

- 25 -

the construct had retained binding properties of the native neurotoxin.

### Example 6

#### 5      **Assay of the biological activity of constructs – measurement of the Mn-SOD activity.**

Mn-SOD activity in samples and constructs was measured by a modification (Brehm *et al.* (1991) Appl. Microbiol. Biotechnol., 36,358-363) of the procedure described by McCord and Fridovich (J. Biol. Chem. 10      (1969), 244, 6049-6055). Aliquots (20 $\mu$ l) of samples or constructs containing Mn-SOD were added to 1ml of 0.05M potassium phosphate buffer pH 7.5 containing  $1 \times 10^{-4}$  M EDTA,  $2.5 \times 10^{-5}$  M ferricytochrome C and  $7 \times 10^{-3}$  M sodium xanthine in a thermostatted cuvette at 30°C. 15      Sufficient xanthine oxidase was added to produce a rate of reduction of the ferricytochrome C at 550nm of approx. 0.1 absorbance units/minute in the absence of Mn-SOD. Under these conditions the amount Mn-SOD that was required to reduce the rate of reduction of ferricytochrome C by 50% was defined as one unit of activity.

20      Using such assays the Mn-SOD activity within constructs was assessed.

### Example 7

#### 25      **Demonstration of the targeting of Mn-SOD to the mitochondria of neuronal cells by constructs of the invention**

Mn-SOD construct containing the translocation and targeting domains derived from botulinum type A neurotoxin was incubated at various 30      concentrations (0.01-10 $\mu$ M final concentration) with a neuroblastoma cell line NG108. Incubations were carried out over a 6h period or overnight at 37°C. In some experiments, construct radiolabelled with  $^{125}$ I iodine was

- 26 -

used. After incubation with the construct cells, were removed from culture  
flasks by gentle scraping and centrifuged at 200 x g. Cells were then  
resuspended in breaking buffer (0.6M mannitol, 20mM hepes pH 7.4 and  
1mM phenylmethylsulphonyl chloride) and homogenised in a Dounce  
homogeniser. The homogenate was centrifuged at 200 x g for 5min and  
then the supernatant fluid recovered and centrifuged at 8000 x g for  
10min. The 200 x g pellet (nuclear fraction) were pooled and resuspended  
in phosphate buffered saline. The 8000 x g pellets (mitochondrial fraction)  
were also pooled and resuspended in phosphate buffered saline. The  
supernatant fluid was saved and used to represent the cytosolic fraction.

Analysis of the sub-cellular distribution of Mn-SOD was carried out by  
Western blot analysis and, where radiolabelled construct was used, by  
analysis of the <sup>125</sup>I-labelled construct components by  $\gamma$ -counting and by  
autoradiography of cell fractions which had been separated by  
electrophoresis on SDS-polyacrylamide gels. For Western blot analysis,  
proteins in the cell fractions were separated by electrophoreses on SDS-  
polyacrylamide gels and then transferred to nitrocellulose membrane as  
described previously (Towbin *et al.* Proc.(1979) Natl. Acad. Sci. USA, 76,  
4350). The presence of Mn-SOD in protein bands on nitrocellulose  
membranes was assessed by incubation with rabbit anti- Mn-SOD antibody  
followed by washing and incubation with anti-rabbit peroxidase conjugate.  
Addition of peroxidase substrates (3,3',5,5'- tetramethyl benzidine and  
H<sub>2</sub>O<sub>2</sub>) allowed visualisation and quantitation of the Mn-SOD in the various  
sub-cellular protein fractions. An enhanced chemiluminescence system  
(Amersham International) was also used in some experiments to increase  
the sensitivity.

#### Example 8.

#### Formulation of the Mn-SOD construct for clinical use.

- 27 -

In a formulation of the Mn-SOD construct for clinical use, recombinant Mn-SOD construct would be prepared under current Good Manufacturing Procedures. The construct would be transferred, by dialysis, to a solution to give the product stability during freeze-drying. Such a formulation may contain Mn-SOD construct (10 mg/ml) in 5mM HEPES buffer (pH 7.2), 50mM NaCl, 1% lactose. The solution, after sterile filtration, would be aliquotted, freeze-dried and stored under nitrogen at -20°C.

#### Example 9.

##### Use of an Mn-SOD construct to treat stroke.

In a typical case of a middle aged or elderly man diagnosed as suffering from stroke, treatment with an Mn-SOD construct would begin immediately, ideally within 6 hours of the stroke occurring. Doses of the Mn-SOD construct (e.g. 100mg) reconstituted in a sterile saline solution would be administered intravenously. Further doses of the construct would be administered daily for 5-10 days. Such a patient would be expected to display reduced levels of ischaemia/reperfusion damage as assessed by magnetic resonance imaging compared to a similarly affected patient receiving no treatment. Relative improvements to muscle strength and co-ordination (MRC motor score) would be expected to be observed over the subsequent 12 month period.

#### Example 10.

##### Method to Demonstrate Neuroprotection of Cells by MnSOD-Heavy Chain Conjugates

Neuroblastoma cell line NG108-15 (*Nature* (1998) 336:p185) were seeded at a density of  $3 \times 10^4$  cells/ml in 96 well microtitre plates coated with poly-D-lysine. Plates were grown for 3 days at 37°C in a CO<sub>2</sub> incubator (5% CO<sub>2</sub>).

- 28 -

95% air). MnSOD or leader-MnSOD conjugates were prepared with purified heavy chain of botulinum neurotoxin serotype A (BoNT/A HC) as described. The concentration of SOD was estimated and the conjugate diluted to give the specified amount of conjugate in a total volume of 200 $\mu$ l serum free medium. Conjugate was added to wells in the presence or absence of 56mM KCl, 2mM CaCl<sub>2</sub>. The cells were incubated with conjugate for 1 hour. The conjugate was replaced with either serum free medium or serum free medium containing 50 $\mu$ M duroquinone and incubated at 37°C for 4 hours in the CO<sub>2</sub> incubator to induce oxidative stress. The media was removed after 4 hours and replaced with the dye 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at a final concentration of 0.25mg/ml in serum free medium and incubated for 2 hours (according to the method of Mattson, MP, *et al* (1995) *Methods in Cell Biology* 46:187-216) The conversion of MTT to formazan dye crystals has been shown to be related to mitochondrial respiratory chain activity (Musser, DA, and Oseroff, AR (1994) *Journal of Immunology* 59:621-626). MTT was removed and crystals solubilised with dimethylsulfoxide (DMSO). Absorbance at 570nm was measured using a Labsystems Multiskan Plus MkII spectrophotometer and the results shown in Figure 5.

Figure 5 shows that a construct of the invention was able to protect neuronal cells against the mitochondrial - focused oxidative stress produced by duroquinone.

#### Example 11.

##### Preparation and purification of recombinant MnSOD and leader sequence MnSOD

Standard molecular biology protocols were used for all genetic manipulations (Sambrook *et al* 1989, Molecular cloning; A laboratory manual. Second Edition, Cold Spring Harbor Laboratory Press, New York.).

- 29 -

The MnSOD gene from either *B. stearrowthermophilus* or *B. caldovenax* was amplified by PCR to engineer a *Bam*HI site (resulting in the replacement of nucleotides 1-15). A synthetic oligonucleotide corresponding to the mitochondrial leader sequence of human MnSOD (bases 1-81 of the human gene) was subcloned into the *Bam*HI site to generate leader-MnSOD. PCR was used to add a Factor Xa cleavage site immediately adjacent to the methionine at the start of the leader sequence. Similarly a Factor Xa cleavage site was engineered immediately adjacent to the methionine at the start of the native MnSOD gene. Constructs were sequenced to confirm the presence of the correct sequence. Constructs for expression were subcloned into the expression vector pET28a (Novagen Inc, Madison, WI) as an *Eco*RI fragment and the orientation of the fragments checked. Clones with confirmed sequences were used to transform expression host *E. coli* BL21 (DE3) (Studier and Moffatt 1986 *Journal of Molecular Biology* **189**:113-130). Examples 2 and 3 above provide detailed methods.

The recombinant proteins expressed from pET28a contain amino-terminal histidine (6-His) and T7 peptide tags allowing proteins to be purified by affinity chromatography on either a  $\text{Cu}^{2+}$  charged metal chelate column or an anti-T7 immunoaffinity column. Incorporation of the Factor Xa site between the peptide tags and the start of either MnSOD or leader MnSOD allows the precise removal of the peptide tags after purification. Cultures of *E. coli* BL21 (DE3) pET28a-MnSOD or BL21 (DE3) pET28a-leader-MnSOD were grown in Terrific Broth containing 30  $\mu\text{g/ml}$  kanamycin and 0.5% (w/v) glucose to an  $\text{OD}_{600}$  of 2.0 and protein expression was induced with 500  $\mu\text{M}$  IPTG for 2 hours. Cells were lysed by sonication, cell debris pelleted by centrifugation and the supernatant loaded onto a metal chelate column charged with  $\text{Cu}^{2+}$  (Amersham-Pharmacia Biotech, Uppsala, Sweden). After loading proteins on the column and washing, proteins were eluted using imidazole. All buffers were used as specified by manufacturers. Factor Xa cleavage of the eluted protein was carried out according to manufacturers instructions.

- 30 -

The invention thus provides constructs and methods for delivery of SOD to neuronal cells.



CLAIMS

1. A composition for delivery of superoxide dismutase (SOD) to neuronal cells, comprising:-

5 SOD; linked by a cleavable linker to  
a neuronal cell targeting component, comprising a first domain that binds to a neuronal cell and a second domain that translocates the SOD of the composition into the neuronal cell.

10 2. A composition according to Claim 1 for delivery of SOD to mitochondria of neuronal cells wherein the SOD comprises a sequence targeting the SOD to mitochondria in the neuronal cell.

15 3. A composition according to Claim 2 wherein the SOD is a hybrid of Mn-SOD and a sequence targeting the hybrid to mitochondria.

4. A composition according to Claim 2 or 3 wherein the mitochondria targeting sequence is derived from human Mn-SOD.

20 5. A composition according to any of Claims 1-4 wherein the SOD is bacterial SOD or is derived therefrom.

25 6. A composition according to any of Claims 1 to 5 wherein the first domain is selected from (a) neuronal cell binding domains of clostridial toxins; and (b) fragments, variants and derivatives of the domains in (a) that substantially retain the neuronal cell binding activity of the domains of (a).

30 7. A composition according to any Claims 1 to 6 wherein the second domain is selected from (a) domains of clostridial neurotoxins that translocate polypeptide sequences into cells, and (b) fragments, variants and derivatives of the domains of (a) that substantially retain the

- 32 -

translocating activity of the domains of (a).

8. A composition according to any of Claims 1 to 7 wherein the linker is a disulphide bridge.

5

9. A pharmaceutical composition for treatment of oxidative damage to neuronal cells comprising a composition according to any of Claims 1 to 8 and a pharmaceutically acceptable carrier.

10

10. A method of delivering SOD to a neuronal cell comprising administering a composition according to Claim 9.

11. A method according to Claim 10 comprising injecting the composition.

15

12. A method of making a composition according to any of Claims 1 to 8 comprising chemically linking SOD, a linker and a neuronal cell targeting component.

20

13. A method of making a composition according to any of Claims 1 to 8 comprising expressing a DNA that codes for a polypeptide having SOD activity, a linker, and a neuronal cell targeting component.

25

14. A method according to claim 13 wherein the polypeptide further comprises a purification sequence and the method further comprises purifying the polypeptide and then cleaving the polypeptide to remove the purification sequence to leave SOD, the linker and the neuronal cell targeting component.

30

15. A composition for delivery of a therapeutic agent to neuronal cells, comprising:-

- 33 -

the therapeutic agent; linked by a cleavable linker to a neuronal cell targeting component, comprising a first domain that binds to a neuronal cell and a second domain that translocates the therapeutic agent of the composition into the neuronal cell.

5

16. A polypeptide comprising a bacterial SOD or derivative thereof and a sequence for targeting the polypeptide to a human mitochondria.

10

17. A polypeptide according to Claim 16 wherein the SOD is from *Bacillus*.

18. A polypeptide according to Claim 16 or 17 which is a fusion protein.

19. A nucleotide encoding the polypeptide of any of Claims 16-18.

15

20. A vector comprising the nucleotide of Claim 19.

21. A method of making a polypeptide according to any of Claims 16-18 comprising expressing the nucleotide sequence of Claim 19.

20

22. A cell comprising the nucleotide sequence of Claim 19 or the vector of Claim 20.

1/4

FIG. 1

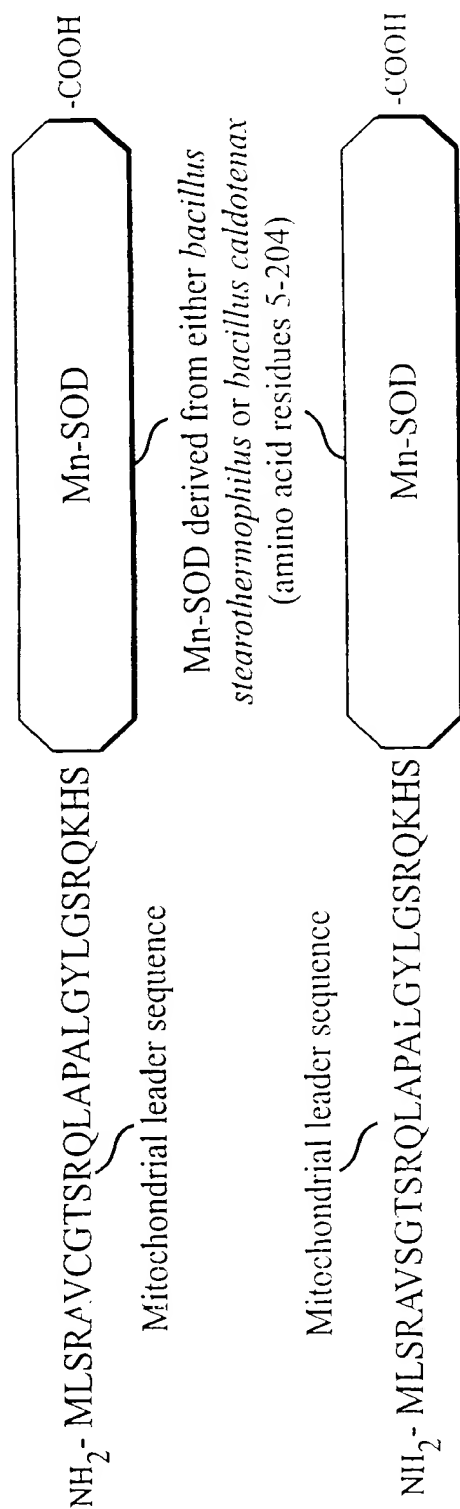
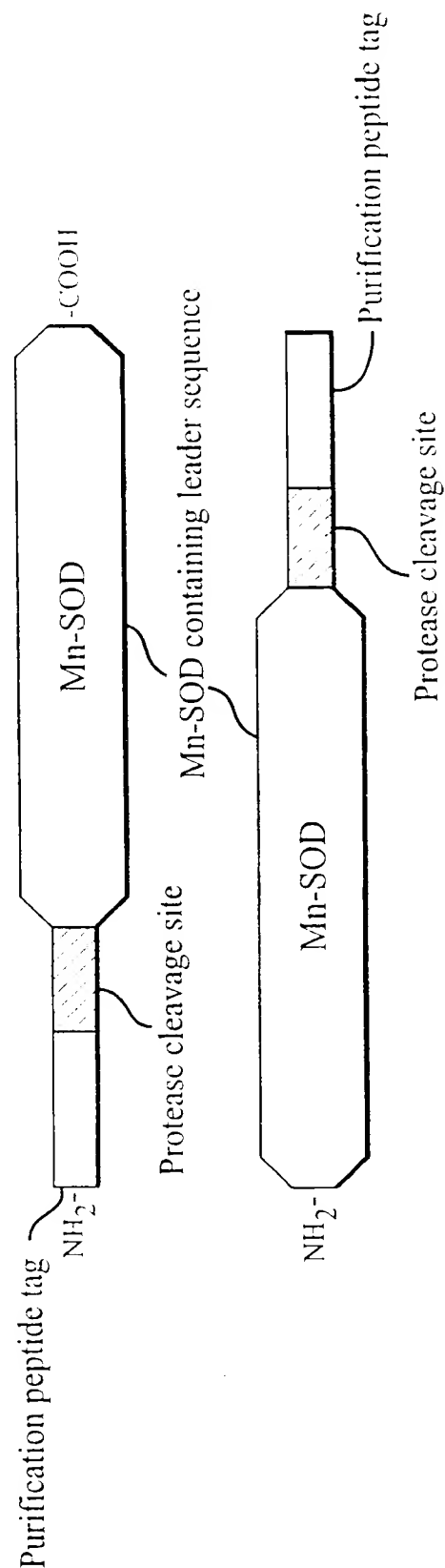
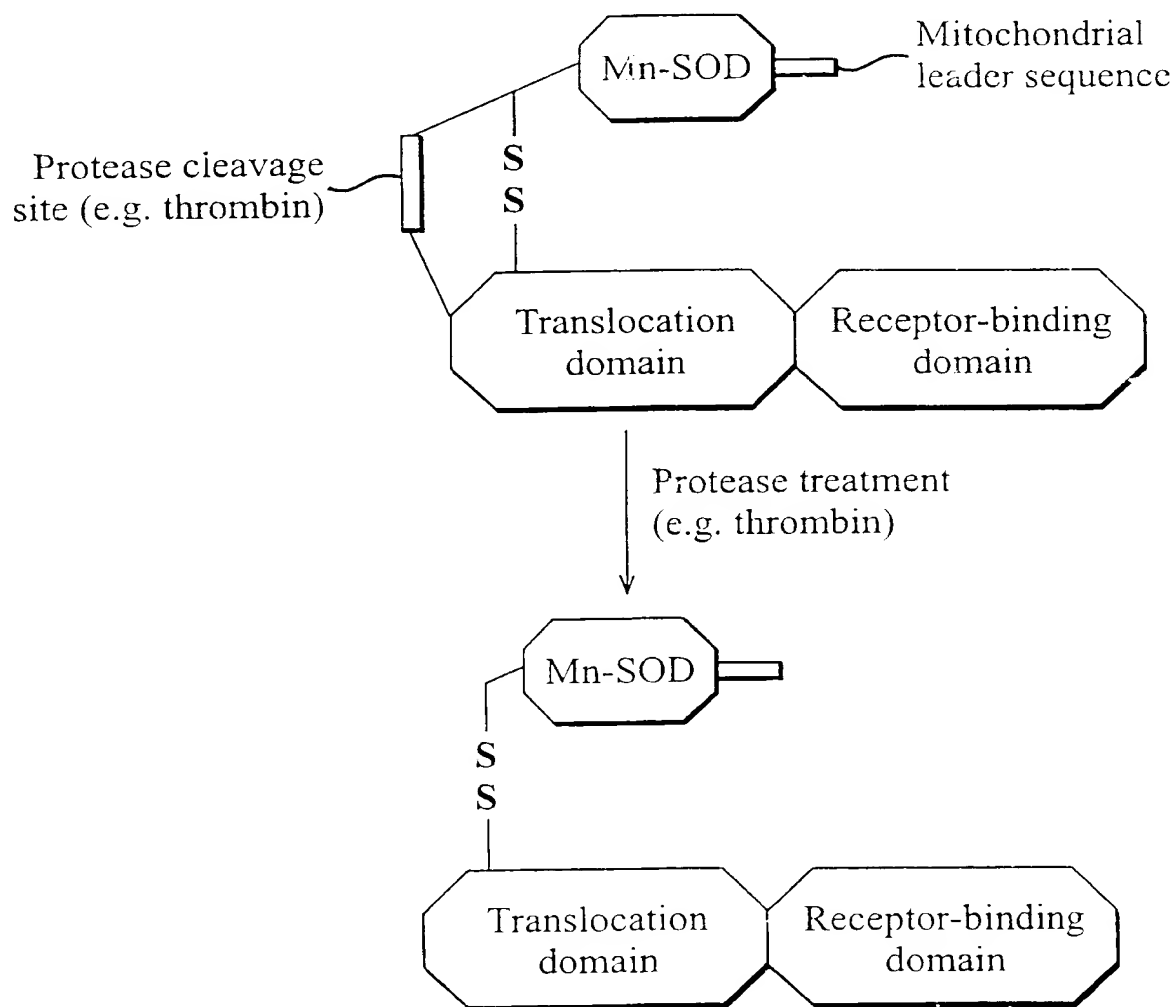


FIG. 2



2/4

FIG. 3



3/4

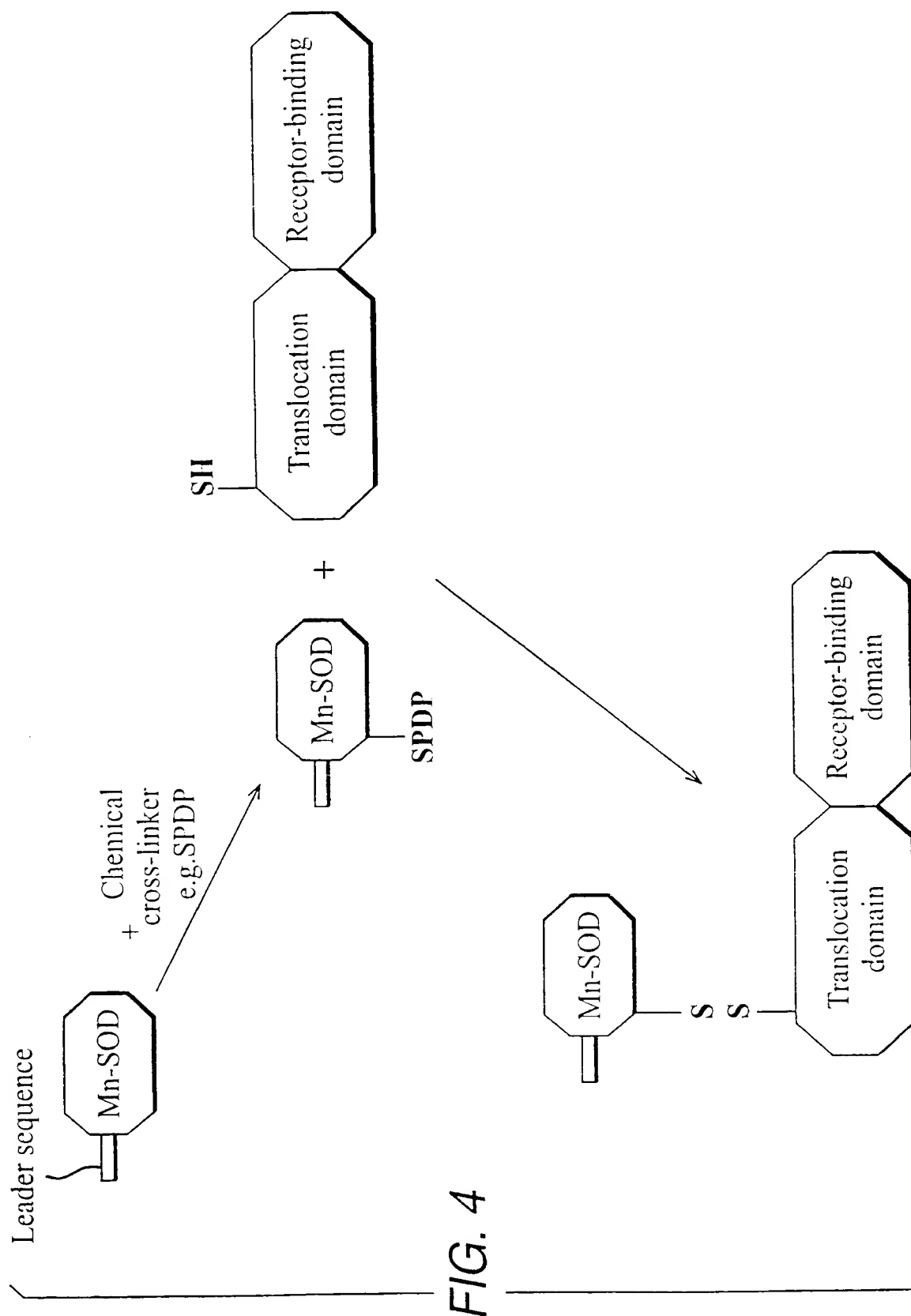
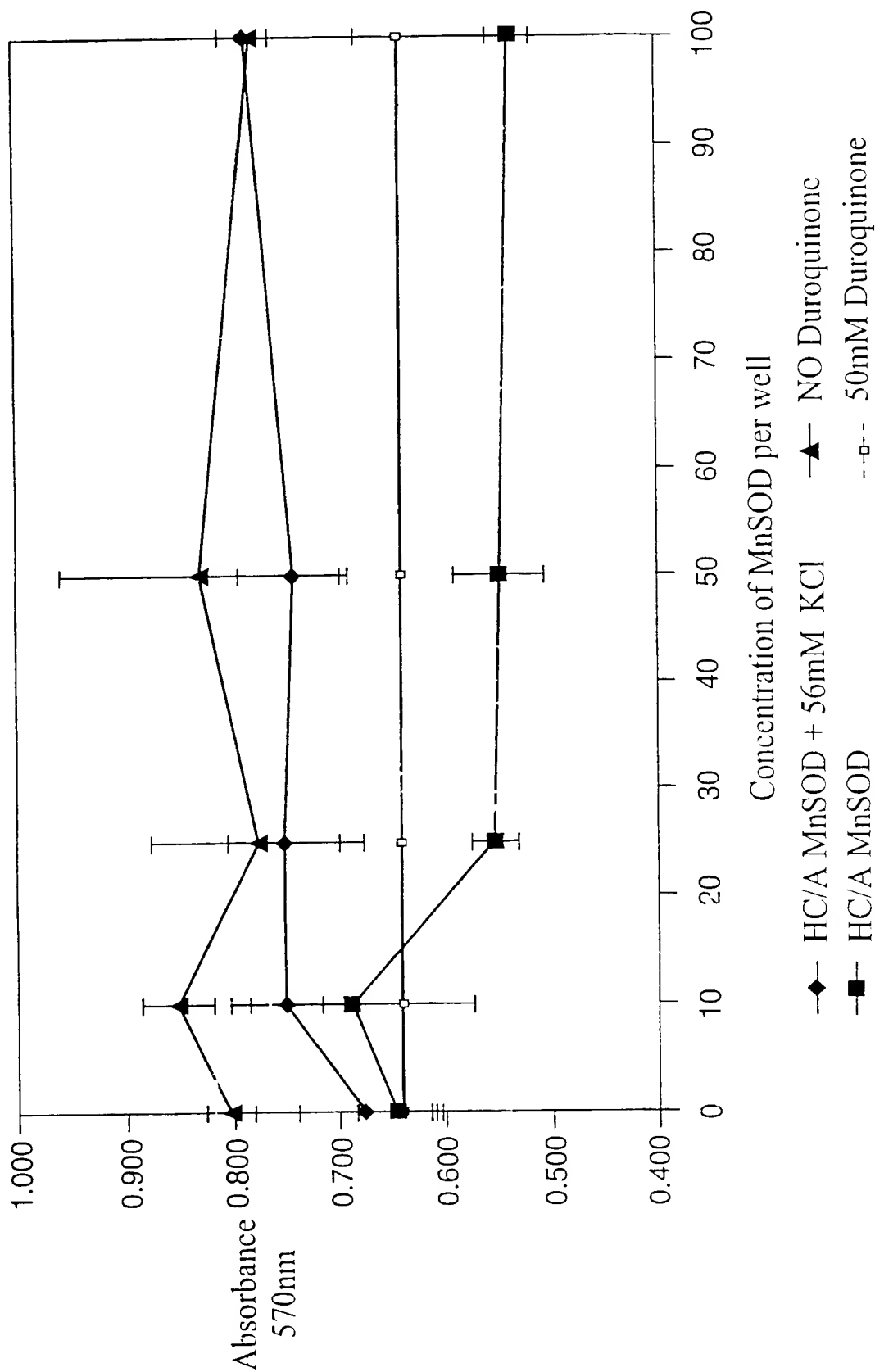


FIG. 4

4/4

**FIG. 5** Demonstration of protective effects of HC/A Mnsod on NG108 cells subjected to oxidative stress by the addition of 50 $\mu$ M duroquinone for 4 hours.



- 1 -

## SEQUENCE LISTING

5 <110> Microbiological Research Authority  
 HALLIS, Bassam  
 SILMAN, Nigel  
 SHONE, Clifford Charles  
 SUTTON, John Mark

10 <120> Delivery of Superoxide Dismutase to Neuronal Cells  
 <130> 20994-SOD-heavy chain conjugates

15 <140>  
 <141>

<150> GB 9824282.9  
 <151> 1998-11-05

20 <160> 11  
 <170> PatentIn Ver. 2.1

25 <210> 1  
 <211> 204  
 <212> PRT  
 <213> Bacillus caldotenax

30 <400> 1  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15  
 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30

35 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45

40 Leu Gln Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Glu Ala Leu  
 50 55 60

Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
 65 70 75 80

45 Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
 85 90 95

Pro Thr Gly Glu Leu Ala Glu Ala Ile Asn Lys Lys Phe Gly Ser Phe  
 100 105 110

50 Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
 115 120 125

55 Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Glu Ile Thr  
 130 135 140

Ser Thr Pro Asn Gln Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
 145 150 155 160

60 Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Gln Asn  
 165 170 175

Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Ile Val Asn Trp Asp  
 180 185 190

65 Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys  
 195 200



- 2 -

5 <210> 2  
 <211> 204  
 <212> PRT  
 <213> *Bacillus stearothermophilus*

10 <400> 2  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15  
 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30  
 15 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45  
 Leu Gln Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Glu Ala Leu  
 50 55 60  
 20 Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
 65 70 75 80  
 25 Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
 85 90 95  
 Pro Thr Gly Glu Leu Ala Asp Ala Ile Asn Lys Lys Phe Gly Ser Phe  
 100 105 110  
 30 Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
 115 120 125  
 Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Glu Ile Thr  
 130 135 140  
 35 Ser Thr Pro Asn Gln Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
 145 150 155 160  
 40 Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Gln Asn  
 165 170 175  
 Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Val Val Asn Trp Asp  
 180 185 190  
 45 Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys  
 195 200

50 <210> 3  
 <211> 1067  
 <212> PRT  
 <213> Artificial Sequence

55 <220>  
 <223> Description of Artificial Sequence:construct

60 <400> 3  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15  
 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30  
 65 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45

- 3 -

	Leu	Gln	Asn	Lys	Ser	Leu	Glu	Glu	Leu	Leu	Ser	Asn	Leu	Glu	Ala	Leu
	50						55					60				
5	Pro	Glu	Ser	Ile	Arg	Thr	Ala	Val	Arg	Asn	Asn	Gly	Gly	Gly	His	Ala
	65					70				75						80
	Asn	His	Ser	Leu	Phe	Trp	Thr	Ile	Leu	Ser	Pro	Asn	Gly	Gly	Gly	Glu
					85					90					95	
10	Pro	Thr	Gly	Glu	Leu	Ala	Asp	Ala	Ile	Asn	Lys	Lys	Phe	Gly	Ser	Phe
				100					105					110		
	Thr	Ala	Phe	Lys	Asp	Glu	Phe	Ser	Lys	Ala	Ala	Ala	Gly	Arg	Phe	Gly
			115					120					125			
15	Ser	Gly	Trp	Ala	Trp	Leu	Val	Val	Asn	Asn	Gly	Glu	Leu	Glu	Ile	Thr
		130					135					140				
20	Ser	Thr	Pro	Asn	Gln	Asp	Ser	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile
	145					150					155					160
	Leu	Gly	Leu	Asp	Val	Trp	Glu	His	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn
					165					170					175	
25	Arg	Arg	Pro	Glu	Tyr	Ile	Ala	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp
				180					185					190		
	Glu	Val	Ala	Lys	Arg	Tyr	Ser	Glu	Ala	Lys	Ala	Lys	Gln	Arg	Ser	Cys
			195					200					205			
30	Gly	Leu	Val	Pro	Arg	Gly	Ser	Gly	Pro	Gly	Ser	Ala	Leu	Asn	Asp	Leu
		210					215					220				
35	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp
	225					230					235					240
	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr
				245						250					255	
40	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln
				260					265					270		
	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile
			275					280					285			
45	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn
		290					295					300				
50	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr
	305					310					315					320
	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg
					325					330					335	
55	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg
				340					345					350		
	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala
			355					360					365			
60	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp
		370					375					380				
65	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp
	385					390					395					400
	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn

- 4 -

	405								410				415			
5	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala
				420					425					430		
	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly
			435					440					445			
10	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln
		450					455					460				
	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val
	465					470					475				480	
15	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile
				485						490					495	
	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu
20				500					505					510		
	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu
			515					520					525			
25	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu
		530					535					540				
	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn
	545					550					555					560
30	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val
					565					570					575	
	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys
35				580					585					590		
	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu
			595					600					605			
40	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu
		610					615					620				
	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr
	625					630					635					640
45	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser
					645					650					655	
	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly
50				660					665					670		
	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe
			675					680					685			
55	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val
		690					695					700				
	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile
	705					710					715					720
60	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile
					725					730					735	
	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly
				740					745					750		
65	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val
				755				760					765			

- 5 -

	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	
	770						775					780					
5	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	
	785					790					795				800		
	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	
					805					810					815		
10	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	
				820					825					830			
	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	
			835					840					845				
15	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	
	850						855					860					
	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	
20	865					870					875					880	
	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	
				885						890					895		
25	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	
				900					905					910			
	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	
30			915					920					925				
	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	
	930						935					940					
35	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	
	945					950					955					960	
	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	
					965					970					975		
40	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	
				980					985					990			
	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	
45			995				1000						1005				
	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	
	1010						1015					1020					
50	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	
	1025					1030					1035				1040		
	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	
				1045						1050					1055		
55	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu						
			1060						1065								
60	<210> 4																
	<211> 1070																
	<212> PRT																
	<213> Artificial Sequence																
65	<220>																
	<223> Description of Artificial Sequence:construct																

- 6 -

<400> 4  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15

5 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30

10 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45

Leu Gln Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Glu Ala Leu  
 50 55 60

15 Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
 65 70 75 80

Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
 85 90 95

20 Pro Thr Gly Glu Leu Ala Asp Ala Ile Asn Lys Lys Phe Gly Ser Phe  
 100 105 110

Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
 115 120 125

25 Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Glu Ile Thr  
 130 135 140

30 Ser Thr Pro Asn Gln Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
 145 150 155 160

Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Gln Asn  
 165 170 175

35 Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Val Val Asn Trp Asp  
 180 185 190

Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys Gln Arg Ser Cys  
 195 200 205

40 Gly Leu Val Pro Arg Gly Ser Gly Pro Gly Ser Lys Ala Pro Gly Ile  
 210 215 220

45 Cys Ile Asp Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn  
 225 230 235 240

Ser Phe Ser Asp Asp Leu Ser Lys Asn Glu Arg Ile Glu Tyr Asn Thr  
 245 250 255

50 Gln Ser Asn Tyr Ile Glu Asn Asp Phe Pro Ile Asn Glu Leu Ile Leu  
 260 265 270

Asp Thr Asp Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu  
 275 280 285

55 Ser Leu Thr Asp Phe Asn Val Asp Val Pro Val Tyr Glu Lys Gln Pro  
 290 295 300

60 Ala Ile Lys Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu  
 305 310 315 320

Tyr Ser Gln Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser  
 325 330 335

65 Ser Phe Asp Asp Ala Leu Leu Phe Ser Asn Lys Val Tyr Ser Phe Phe  
 340 345 350

- 7 -

	Ser	Met	Asp	Tyr	Ile	Lys	Thr	Ala	Asn	Lys	Val	Val	Glu	Ala	Gly	Leu
			355					360					365			
5	Phe	Ala	Gly	Trp	Val	Lys	Gln	Ile	Val	Asn	Asp	Phe	Val	Ile	Glu	Ala
		370					375					380				
	Asn	Lys	Ser	Asn	Thr	Met	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val
		385				390					395					400
10	Pro	Tyr	Ile	Gly	Leu	Ala	Leu	Asn	Val	Gly	Asn	Glu	Thr	Ala	Lys	Gly
					405					410					415	
	Asn	Phe	Glu	Asn	Ala	Phe	Glu	Ile	Ala	Gly	Ala	Ser	Ile	Leu	Leu	Glu
				420					425					430		
15	Phe	Ile	Pro	Glu	Leu	Leu	Ile	Pro	Val	Val	Gly	Ala	Phe	Leu	Leu	Glu
			435					440					445			
	Ser	Tyr	Ile	Asp	Asn	Lys	Asn	Lys	Ile	Ile	Lys	Thr	Ile	Asp	Asn	Ala
20			450				455					460				
	Leu	Thr	Lys	Arg	Asn	Glu	Lys	Trp	Ser	Asp	Met	Tyr	Gly	Leu	Ile	Val
		465				470					475					480
25	Ala	Gln	Trp	Leu	Ser	Thr	Val	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	Lys	Glu
					485					490					495	
	Gly	Met	Tyr	Lys	Ala	Leu	Asn	Tyr	Gln	Ala	Gln	Ala	Leu	Glu	Glu	Ile
30				500					505					510		
	Ile	Lys	Tyr	Arg	Tyr	Asn	Ile	Tyr	Ser	Glu	Lys	Glu	Lys	Ser	Asn	Ile
			515					520					525			
35	Asn	Ile	Asp	Phe	Asn	Asp	Ile	Asn	Ser	Lys	Leu	Asn	Glu	Gly	Ile	Asn
		530					535					540				
	Gln	Ala	Ile	Asp	Asn	Ile	Asn	Asn	Phe	Ile	Asn	Gly	Cys	Ser	Val	Ser
		545				550					555					560
40	Tyr	Leu	Met	Lys	Lys	Met	Ile	Pro	Leu	Ala	Val	Glu	Lys	Leu	Leu	Asp
					565					570					575	
	Phe	Asp	Asn	Thr	Leu	Lys	Lys	Asn	Leu	Leu	Asn	Tyr	Ile	Asp	Glu	Asn
45				580					585					590		
	Lys	Leu	Tyr	Leu	Ile	Gly	Ser	Ala	Glu	Tyr	Glu	Lys	Ser	Lys	Val	Asn
			595					600					605			
50	Lys	Tyr	Leu	Lys	Thr	Ile	Met	Pro	Phe	Asp	Leu	Ser	Ile	Tyr	Thr	Asn
		610					615					620				
	Asp	Thr	Ile	Leu	Ile	Glu	Met	Phe	Asn	Lys	Tyr	Asn	Ser	Glu	Ile	Leu
		625				630					635					640
55	Asn	Asn	Ile	Ile	Leu	Asn	Leu	Arg	Tyr	Lys	Asp	Asn	Asn	Leu	Ile	Asp
					645					650					655	
	Leu	Ser	Gly	Tyr	Gly	Ala	Lys	Val	Glu	Val	Tyr	Asp	Gly	Val	Glu	Leu
60				660					665					670		
	Asn	Asp	Lys	Asn	Gln	Phe	Lys	Leu	Thr	Ser	Ser	Ala	Asn	Ser	Lys	Ile
			675					680					685			
65	Arg	Val	Thr	Gln	Asn	Gln	Asn	Ile	Ile	Phe	Asn	Ser	Val	Phe	Leu	Asp
		690					695					700				
	Phe	Ser	Val	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Lys	Asn	Asp	Gly

7

- 23 -

&lt;220&gt;

<223> Description of Artificial Sequence: modified human  
mitochondrial leader sequence

5

&lt;400&gt; 11

Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
1 5 10 15

10

Leu Gly Tyr Leu Gly Ser Arg Gln  
20



- 22 -

Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
                     20                    25                    30

5    Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
                     35                    40                    45

Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
                     50                    55                    60

10    Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
                     65                    70                    75                    80

Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
                     85                    90                    95

15    Val Arg Asn Asn Gly Gly Gly His Ala Asn His Ser Leu Phe Trp Thr  
                     100                    105                    110

20    Ile Leu Ser Pro Asn Gly Gly Gly Glu Pro Thr Gly Glu Leu Ala Asp  
                     115                    120                    125

Ala Ile Asn Lys Lys Phe Gly Ser Phe Thr Ala Phe Lys Asp Glu Phe  
                     130                    135                    140

25    Ser Lys Ala Ala Ala Gly Arg Phe Gly Ser Gly Trp Ala Trp Leu Val  
                     145                    150                    155                    160

Val Asn Asn Gly Glu Leu Glu Ile Thr Ser Thr Pro Asn Gln Asp Ser  
                     165                    170                    175

30    Pro Ile Met Glu Gly Lys Thr Pro Ile Leu Gly Leu Asp Val Trp Glu  
                     180                    185                    190

35    His Ala Tyr Tyr Leu Lys Tyr Gln Asn Arg Arg Pro Glu Tyr Ile Ala  
                     195                    200                    205

Ala Phe Trp Asn Val Val Asn Trp Asp Glu Val Ala Lys Arg Tyr Ser  
                     210                    215                    220

40    Glu Ala Lys Ala Lys  
                     225

45    <210> 10  
       <211> 24  
       <212> FRT  
       <213> Artificial Sequence

50    <220>  
       <223> Description of Artificial Sequence: modified human  
                     mitochondrial leader sequence

55    <400> 10  
       Met Leu Ser Arg Ala Val Ser Gly Thr Ser Arg Gln Leu Ala Pro Ala  
                     1                    5                    10                    15

60    Leu Gly Tyr Leu Gly Ser Arg Gln  
                     20

65    <210> 11  
       <211> 24  
       <212> FRT  
       <213> Artificial Sequence

- 21 -

Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser Arg Ile Tyr Ile Asn  
 820 825 830  
 5 Gly Asn Leu Ile Asp Glu Lys Ser Ile Ser Asn Leu Gly Asp Ile His  
 835 840 845  
 Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly Cys Asn Asp Thr Arg  
 850 855 860  
 10 Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe Asp Thr Glu Leu Gly Lys  
 865 870 875 880  
 Thr Glu Ile Glu Thr Leu Tyr Ser Asp Glu Pro Asp Pro Ser Ile Leu  
 885 890 895  
 15 Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asn Lys Arg Tyr Tyr Leu  
 900 905 910  
 Leu Asn Leu Leu Arg Thr Asp Lys Ser Ile Thr Gln Asn Ser Asn Phe  
 915 920 925  
 20 Leu Asn Ile Asn Gln Gln Arg Gly Val Tyr Gln Lys Pro Asn Ile Phe  
 930 935 940  
 25 Ser Asn Thr Arg Leu Tyr Thr Gly Val Glu Val Ile Ile Arg Lys Asn  
 945 950 955 960  
 Gly Ser Thr Asp Ile Ser Asn Thr Asp Asn Phe Val Arg Lys Asn Asp  
 965 970 975  
 30 Leu Ala Tyr Ile Asn Val Val Asp Arg Asp Val Glu Tyr Arg Leu Tyr  
 980 985 990  
 35 Ala Asp Ile Ser Ile Ala Lys Pro Glu Lys Ile Ile Lys Leu Ile Arg  
 995 1000 1005  
 Thr Ser Asn Ser Asp Asn Ser Leu Gly Gln Ile Ile Val Met Asp Ser  
 1010 1015 1020  
 40 Ile Gly Asn Asn Cys Thr Met Asn Phe Gln Asn Asn Asn Gly Gly Asn  
 1025 1030 1035 1040  
 Ile Gly Leu Leu Gly Phe His Ser Asn Asn Leu Val Ala Ser Ser Trp  
 1045 1050 1055  
 45 Tyr Tyr Asn Asn Ile Arg Lys Asn Thr Ser Ser Asn Gly Cys Phe Trp  
 1060 1065 1070  
 50 Ser Phe Ile Ser Lys Glu His Gly Trp Gln Glu Asn  
 1075 1080  
 55 <210> 9  
 <211> 329  
 <212> PRT  
 <213> Artificial Sequence  
 60 <220>  
 <223> Description of Artificial Sequence: polypeptide  
 comprising a mitochondrial leader from human Wn90L  
 and E. Stearothermophilus SOD  
 65 <400> 9  
 Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
 1 5 10 15

- 20 -

	450	455	460
5	Leu 465	Leu Ile Pro Thr Ile 470	Leu Val Phe Thr Ile 475
10	Ser Ser Glu Asn 485	Lys Asn Lys Ile Ile 490	Lys Ser Phe Ile Gly 480
15	Met Glu Arg Glu Thr 500	Lys Trp Lys Glu Ile Tyr Ser Trp 510	Val Ser
20	Asn Trp Leu Thr Arg 515	Ile Asn Thr Gln Phe Asn Lys Arg 525	Lys Glu Gln
25	Met Tyr Gln Ala Leu 530	Gln Asn Gln Val Asp Ala 540	Ile Lys Thr Val Ile
30	Glu Tyr Lys Tyr Asn 550	Asn Tyr Thr Ser Asp Glu 555	Arg Asn Arg Leu Glu 560
35	Ser Glu Tyr Asn 565	Ile Asn Asn Ile Arg Glu 570	Glu Leu Asn Lys Lys Val 575
40	Ser Leu Ala Met Glu 580	Asn Ile Glu Arg Phe 585	Ile Thr Glu Ser Ser Ile 590
45	Phe Tyr Leu Met Lys 595	Leu Ile Asn Glu Ala Lys 605	Val Ser Lys Leu Arg 605
50	Glu Tyr Asp Glu Gly Val 615	Lys Glu Tyr Leu Leu Asp 620	Tyr Ile Ser Glu 625
55	His Arg Ser Ile Leu 630	Gly Asn Ser Val Gln Glu 635	Leu Asn Asp Leu Val 640
60	Thr Ser Thr Leu Asn 645	Asn Ser Ile Pro Phe Glu 650	Leu Ser Ser Tyr Thr 655
65	Asn Asp Lys Ile Leu 660	Ile Leu Tyr Phe Asn Lys 665	Leu Tyr Lys Lys Ile 670
70	Lys Asp Asn Ser Ile Leu 675	Asp Met Arg Tyr Glu Asn 685	Asn Lys Phe Ile 685
75	Asp Ile Ser Gly Tyr Gly 695	Ser Asn Ile Ser Ile Asn 700	Gly Asp Val Tyr 705
80	Ile Tyr Ser Thr Asn 710	Arg Asn Gln Phe Gly 715	Ile Tyr Ser Ser Lys Pro 720
85	Ser Glu Val Asn 725	Ile Ala Gln Asn Asn Asp 730	Ile Ile Tyr Asn Gly Arg 735
90	Tyr Gln Asn Phe 740	Ser Ile Ser Phe Trp Val Arg 745	Ile Pro Lys Tyr Phe 750
95	Asn Lys Val Asn Leu 755	Asn Asn Glu Tyr Thr Ile 760	Ile Asp Cys Ile Arg 765
100	Asn Asn Asn Ser Gly 770	Trp Lys Ile Ser Leu Asn 775	Tyr Asn Lys Ile Ile 780
105	Trp Thr Leu Gln Asp 785	Thr Ala Gly Asn Asn Gln 795	Lys Leu Val Phe Asn 800
110	Tyr Thr Gln Met 805	Ile Ser Ile Ser Asp Tyr 810	Ile Asn Lys Trp Ile Phe 815

- 19 -

	Val	Arg	Asn	Asn	Gly	Gly	Gly	His	Ala	Asn	His	Ser	Leu	Phe	Trp	Thr
			100						105					110		
5	Ile	Leu	Ser	Pro	Asn	Gly	Gly	Gly	Glu	Pro	Thr	Gly	Glu	Leu	Ala	Asp
			115					120					125			
	Ala	Ile	Asn	Lys	Lys	Phe	Gly	Ser	Phe	Thr	Ala	Phe	Lys	Asp	Glu	Phe
	130						135					140				
10	Ser	Lys	Ala	Ala	Ala	Gly	Arg	Phe	Gly	Ser	Gly	Trp	Ala	Trp	Leu	Val
	145					150					155				160	
	Val	Asn	Asn	Gly	Glu	Leu	Glu	Ile	Thr	Ser	Thr	Pro	Asn	Gln	Asp	Ser
				165						170					175	
15	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile	Leu	Gly	Leu	Asp	Val	Trp	Glu
			180						185					190		
	His	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn	Arg	Arg	Pro	Glu	Tyr	Ile	Ala
20			195					200					205			
	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp	Glu	Val	Ala	Lys	Arg	Tyr	Ser
	210						215					220				
25	Glu	Ala	Lys	Ala	Lys	Gln	Arg	Ser	Cys	Gly	Leu	Val	Pro	Arg	Gly	Ser
	225					230					235				240	
	Gly	Pro	Gly	Ser	Lys	Ala	Pro	Pro	Arg	Leu	Cys	Ile	Arg	Val	Asn	Asn
				245						250					255	
30	Arg	Glu	Leu	Phe	Phe	Val	Ala	Ser	Glu	Ser	Ser	Tyr	Asn	Glu	Asn	Asp
			260						265					270		
	Ile	Asn	Thr	Pro	Lys	Glu	Ile	Asp	Asp	Thr	Thr	Asn	Leu	Asn	Asn	Asn
35			275					280					285			
	Tyr	Arg	Asn	Asn	Leu	Asp	Glu	Val	Ile	Leu	Asp	Tyr	Asn	Ser	Glu	Thr
	290					295						300				
40	Ile	Pro	Gln	Ile	Ser	Asn	Gln	Thr	Leu	Asn	Thr	Leu	Val	Gln	Asp	Asp
	305					310					315				320	
	Ser	Tyr	Val	Pro	Arg	Tyr	Asp	Ser	Asn	Gly	Thr	Ser	Glu	Ile	Glu	Glu
				325						330					335	
45	His	Asn	Val	Val	Asp	Leu	Asn	Val	Phe	Phe	Tyr	Leu	His	Ala	Gln	Lys
			340						345					350		
	Val	Pro	Glu	Gly	Glu	Thr	Asn	Ile	Ser	Leu	Thr	Ser	Ser	Ile	Asp	Thr
50			355					360					365			
	Ala	Leu	Ser	Glu	Glu	Ser	Gln	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Glu	Phe
	370					375						380				
55	Ile	Asn	Thr	Ile	Asn	Lys	Pro	Val	His	Ala	Ala	Leu	Phe	Ile	Ser	Trp
	385					390					395				400	
	Ile	Asn	Gln	Val	Ile	Arg	Asp	Phe	Thr	Thr	Glu	Ala	Thr	Gln	Lys	Ser
				405						410					415	
60	Thr	Phe	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Val	Val	Pro	Tyr	Val	Gly
			420						425					430		
	Leu	Ala	Leu	Asn	Ile	Gly	Asn	Glu	Val	Gln	Lys	Glu	Asn	Phe	Lys	Glu
65			435					440					445			
	Ala	Phe	Glu	Leu	Leu	Gly	Ala	Gly	Ile	Leu	Leu	Glu	Phe	Val	Pro	Glu

- 18 -

Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly Asn Lys Asn Ser  
 900 905 910  
 5 Tyr Ile Lys Leu Lys Lys Asp Ser Pro Val Gly Glu Ile Leu Thr Arg  
 915 920 925  
 Ser Lys Tyr Asn Gln Asn Ser Lys Tyr Ile Asn Tyr Arg Asp Leu Tyr  
 930 935 940  
 10 Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn Ser Gln Ser Ile  
 945 950 955 960  
 Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr Leu Asp Phe Phe  
 965 970 975  
 15 Asn Leu Asn Gln Glu Trp Arg Val Tyr Thr Tyr Lys Tyr Phe Lys Lys  
 980 985 990  
 20 Glu Glu Glu Lys Leu Phe Leu Ala Pro Ile Ser Asp Ser Asp Glu Phe  
 995 1000 1005  
 Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln Pro Thr Tyr Ser  
 1010 1015 1020  
 25 Lys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr Asp Glu Ile Gly  
 1025 1030 1035 1040  
 Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile Val Phe Glu Glu  
 1045 1050 1055  
 30 Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu Lys Glu Val Lys  
 1060 1065 1070  
 35 Arg Lys Pro Tyr Asn Leu Lys Leu Gly Cys Asn Trp Gln Phe Ile Pro  
 1075 1080 1085  
 Lys Asp Glu Gly Trp Thr Glu  
 1090 1095  
 40  
 <210> 8  
 <211> 1034  
 <212> FRT  
 45 <213> Artificial Sequence  
 <210>  
 <223> Description of Artificial Sequence:construct  
 50 <400> 8  
 Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
 1 5 10 15  
 55 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
 20 25 30  
 Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
 35 40 45  
 60 Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
 50 55 60  
 Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
 65 70 75 80  
 65 Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
 85 90 95

- 17 -

	530	535	540	
5	Tyr Ser Glu Lys Glu Lys Ser Asn Ile Asn Ile Asp Phe Asn Asp Ile 545 550 555 560			
	Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile Asp Asn Ile Asn 565 570 575			
10	Asn Phe Ile Asn Gly Cys Ser Val Ser Tyr Leu Met Lys Lys Met Ile 580 585 590			
	Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn Thr Leu Lys Lys 595 600 605			
15	Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr Leu Ile Gly Ser 610 615 620			
20	Ala Glu Tyr Glu Lys Ser Lys Val Asn Lys Tyr Leu Lys Thr Ile Met 625 630 635 640			
	Pro Phe Asp Leu Ser Ile Tyr Thr Asn Asp Thr Ile Leu Ile Glu Met 645 650 655			
25	Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile Ile Leu Asn Leu 660 665 670			
	Arg Tyr Lys Asp Asn Asn Leu Ile Asp Leu Ser Gly Tyr Gly Ala Lys 675 680 685			
30	Val Glu Val Tyr Asp Gly Val Glu Leu Asn Asp Lys Asn Gln Phe Lys 690 695 700			
35	Leu Thr Ser Ser Ala Asn Ser Lys Ile Arg Val Thr Gln Asn Gln Asn 705 710 715 720			
	Ile Ile Phe Asn Ser Val Phe Leu Asp Phe Ser Val Ser Phe Trp Ile 725 730 735			
40	Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn Tyr Ile His Asn 740 745 750			
	Glu Tyr Thr Ile Ile Asn Cys Met Lys Asn Asn Ser Gly Trp Lys Ile 755 760 765			
45	Ser Ile Arg Gly Asn Arg Ile Ile Trp Thr Leu Ile Asp Ile Asn Gly 770 775 780			
50	Lys Thr Lys Ser Val Phe Phe Glu Tyr Asn Ile Arg Glu Asp Ile Ser 785 790 795 800			
	Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr Asn Asn Leu Asn 805 810 815			
55	Asn Ala Lys Ile Tyr Ile Asn Gly Lys Leu Glu Ser Asn Thr Asp Ile 820 825 830			
	Lys Asp Ile Arg Glu Val Ile Ala Asn Gly Glu Ile Ile Phe Lys Leu 835 840 845			
60	Asp Gly Asp Ile Asp Arg Thr Gln Phe Ile Trp Met Lys Tyr Phe Ser 850 855 860			
65	Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Glu Glu Arg Tyr Lys 865 870 875 880			
	Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp Gly Asn Pro Leu 885 890 895			

- 16 -

	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile	Leu	Gly	Leu	Asp	Val	Trp	Glu
				180					185					190		
5	Hie	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn	Arg	Arg	Pro	Glu	Tyr	Ile	Ala
			195					200					205			
	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp	Glu	Val	Ala	Lys	Arg	Tyr	Ser
		210					215					220				
10	Glu	Ala	Lys	Ala	Lys	Gln	Arg	Ser	Cys	Gly	Leu	Val	Pro	Arg	Gly	Ser
	225					230					235					240
	Gly	Pro	Gly	Ser	Lys	Ala	Pro	Gly	Ile	Cys	Ile	Asp	Val	Asp	Asn	Glu
					245					250					255	
15	Asp	Leu	Phe	Phe	Ile	Ala	Asp	Lys	Asn	Ser	Phe	Ser	Asp	Asp	Leu	Ser
				260					265					270		
	Lys	Asn	Glu	Arg	Ile	Glu	Tyr	Asn	Thr	Gln	Ser	Asn	Tyr	Ile	Glu	Asn
20			275					280					285			
	Asp	Phe	Pro	Ile	Asn	Glu	Leu	Ile	Leu	Asp	Thr	Asp	Leu	Ile	Ser	Lys
		290					295					300				
25	Ile	Glu	Leu	Pro	Ser	Glu	Asn	Thr	Glu	Ser	Leu	Thr	Asp	Phe	Asn	Val
	305					310					315					320
	Asp	Val	Pro	Val	Tyr	Glu	Lys	Gln	Pro	Ala	Ile	Lys	Lys	Ile	Phe	Thr
					325					330					335	
30	Asp	Glu	Asn	Thr	Ile	Phe	Gln	Tyr	Leu	Tyr	Ser	Gln	Thr	Phe	Pro	Leu
				340					345					350		
	Asp	Ile	Arg	Asp	Ile	Ser	Leu	Thr	Ser	Ser	Phe	Asp	Asp	Ala	Leu	Leu
35			355					360					365			
	Phe	Ser	Asn	Lys	Val	Tyr	Ser	Phe	Phe	Ser	Met	Asp	Tyr	Ile	Lys	Thr
		370					375					380				
40	Ala	Asn	Lys	Val	Val	Glu	Ala	Gly	Leu	Phe	Ala	Gly	Trp	Val	Lys	Gln
	385					390					395					400
	Ile	Val	Asn	Asp	Phe	Val	Ile	Glu	Ala	Asn	Lys	Ser	Asn	Thr	Met	Asp
45					405				410						415	
	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val	Pro	Tyr	Ile	Gly	Leu	Ala	Leu
			420					425						430		
	Asn	Val	Gly	Asn	Glu	Thr	Ala	Lys	Gly	Asn	Phe	Glu	Asn	Ala	Phe	Glu
50			435					440					445			
	Ile	Ala	Gly	Ala	Ser	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Leu	Leu	Ile
	450						455					460				
55	Pro	Val	Val	Gly	Ala	Phe	Leu	Leu	Glu	Ser	Tyr	Ile	Asp	Asn	Lys	Asn
	465					470					475					480
	Lys	Ile	Ile	Lys	Thr	Ile	Asp	Asn	Ala	Leu	Thr	Lys	Arg	Asn	Glu	Lys
					485				490						495	
60	Trp	Ser	Asp	Met	Tyr	Gly	Leu	Ile	Val	Ala	Gln	Trp	Leu	Ser	Thr	Val
				500					505					510		
	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	Lys	Glu	Gly	Met	Tyr	Lys	Ala	Leu	Asn
65			515					520					525			
	Tyr	Gln	Ala	Gln	Ala	Leu	Glu	Glu	Ile	Ile	Lys	Tyr	Arg	Tyr	Asn	Ile

- 15 -

Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn  
 980 985 990  
 5 Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro  
 995 1000 1005  
 Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp  
 1010 1015 1020  
 10 Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly  
 1025 1030 1035 1040  
 Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys  
 1045 1050 1055  
 15 Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg  
 1060 1065 1070  
 20 Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly  
 1075 1080 1085  
 Glu Arg Pro Leu  
 1090  
 25  
 <210> 7  
 <211> 1095  
 <212> PRT  
 30 <213> Artificial Sequence  
 <220>  
 <223> Description of Artificial Sequence:construct  
 35 <400> 7  
 Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Glu Leu Ala Pro Ala  
 1 5 10 15  
 40 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
 20 25 30  
 Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
 35 40 45  
 45 Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
 50 55 60  
 Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
 65 70 75 80  
 50 Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
 85 90 95  
 55 Val Arg Asn Asn Gly Gly Gly His Ala Asn His Ser Leu Phe Trp Thr  
 100 105 110  
 Ile Leu Ser Pro Asn Gly Gly Gly Glu Pro Thr Gly Glu Leu Ala Asp  
 115 120 125  
 60 Ala Ile Asn Lys Lys Phe Gly Ser Phe Thr Ala Phe Lys Asp Glu Phe  
 130 135 140  
 Ser Lys Ala Ala Ala Gly Arg Phe Gly Ser Gly Trp Ala Trp Leu Val  
 145 150 155 160  
 65 Val Asn Asn Gly Glu Leu Glu Ile Thr Ser Thr Pro Asn Gln Asp Ser  
 165 170 175



- 14 -

	610					615					620				
5	Thr 625	Leu	Ile	Gly	Gln	Val 630	Asp	Arg	Leu	Lys	Asp 635	Lys	Val	Asn	Thr 640
	Leu	Ser	Thr	Asp 645	Ile	Pro	Phe	Gln	Leu	Ser 650	Lys	Tyr	Val	Asn 655	Gln
10	Arg	Leu	Leu	Ser 660	Thr	Phe	Thr	Glu	Tyr 665	Ile	Lys	Asn	Ile	Asn 670	Thr
	Ser	Ile	Leu	Asn 675	Leu	Arg	Tyr	Glu	Ser 680	Asn	His	Leu	Ile	Asp 685	Ser
15	Arg	Tyr	Ala	Ser	Lys	Ile	Asn 695	Ile	Gly	Ser	Lys	Val 700	Asn	Phe	Pro
	Ile	Asp	Lys	Asn	Gln	Ile	Gln 710	Leu	Phe	Asn	Leu 715	Glu	Ser	Lys	Ile 720
20	Glu	Val	Ile	Leu	Lys 725	Asn	Ala	Ile	Val	Tyr 730	Asn	Ser	Met	Tyr	Asn 735
25	Phe	Ser	Thr	Ser 740	Phe	Trp	Ile	Arg	Ile 745	Pro	Lys	Tyr	Phe	Asn 750	Ile
	Ser	Leu	Asn	Asn 755	Glu	Tyr	Thr	Ile	Ile 760	Asn	Cys	Met	Glu	Asn 765	Ser
30	Gly	Trp	Lys	Val	Ser	Leu	Asn 775	Tyr	Gly	Glu	Ile	Ile 780	Trp	Thr	Gln
	Asp	Thr	Gln	Glu	Ile	Lys	Gln 790	Arg	Val	Val	Phe 795	Lys	Tyr	Ser	Met 800
35	Ile	Asn	Ile	Ser	Asp 805	Tyr	Ile	Asn	Arg	Trp 810	Ile	Phe	Val	Thr	Thr 815
40	Asn	Asn	Arg	Leu	Asn 820	Asn	Ser	Lys	Ile 825	Tyr	Ile	Asn	Gly	Arg 830	Ile
	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu 840	Gly	Asn	Ile	His	Ala	Ser 845	Asn
45	Ile	Met	Phe	Lys	Leu	Asp	Gly 855	Cys	Arg	Asp	Thr	His	Arg	Tyr	Trp 860
	Ile	Lys	Tyr	Phe	Asn	Leu	Phe 870	Asp	Lys	Glu	Leu 875	Asn	Glu	Lys	Ile 880
50	Lys	Asp	Leu	Tyr	Asp 885	Asn	Gln	Ser	Asn	Ser 890	Gly	Ile	Leu	Lys	Phe 895
55	Trp	Gly	Asp	Tyr 900	Leu	Gln	Tyr	Asp	Lys 905	Pro	Tyr	Tyr	Met	Leu 910	Leu
	Tyr	Asp	Pro	Asn 915	Lys	Tyr	Val	Asp 920	Val	Asn	Asn	Val	Gly	Ile 925	Gly
60	Tyr	Met	Tyr	Leu	Lys	Gly	Pro 935	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn 940
	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr 950	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys 955
65	Tyr	Ala	Ser	Gly	Asn 965	Lys	Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Val 975

- 13 -

	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn
				260					265					270		
5	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu
			275					280					285			
	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe
		290					295					300				
10	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile
	305					310					315					320
	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly
					325					330					335	
15	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala
				340					345					350		
	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val
20			355					360					365			
	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser
		370					375					380				
25	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu
	385					390					395					400
	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu
				405						410					415	
30	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr
			420						425					430		
	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe
35			435					440					445			
	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile
		450				455						460				
40	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr
	465					470					475					480
	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser
				485					490						495	
45	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn
			500					505						510		
	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met
50			515					520					525			
	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn
		530					535					540				
55	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe
	545					550					555					560
	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala
				565						570					575	
60	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu
			580						585					590		
	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp
65			595					600					605			
	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly

- 12 -

Asn Leu Val Ala Ser Ser Trp Tyr Tyr Asn Asn Ile Arg Lys Asn Thr  
 1025 1030 1035 1040  
 Ser Ser Asn Gly Cys Phe Trp Ser Phe Ile Ser Lys Glu His Gly Trp  
 1045 1050 1055  
 Gln Glu Asn  
 <210> 6  
 <211> 1092  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Description of Artificial Sequence: construct  
 <400> 6  
 Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
 1 5 10 15  
 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
 20 25 30  
 Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
 35 40 45  
 Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
 50 55 60  
 Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
 65 70 75 80  
 Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
 85 90 95  
 Val Arg Asn Asn Gly Gly Gly His Ala Asn His Ser Leu Phe Trp Thr  
 100 105 110  
 Ile Leu Ser Pro Asn Gly Gly Gly Glu Pro Thr Gly Glu Leu Ala Asp  
 115 120 125  
 Ala Ile Asn Lys Lys Phe Gly Ser Phe Thr Ala Phe Lys Asp Glu Phe  
 130 135 140  
 Ser Lys Ala Ala Ala Gly Arg Phe Gly Ser Gly Trp Ala Trp Leu Val  
 145 150 155 160  
 Val Asn Asn Gly Glu Leu Glu Ile Thr Ser Thr Pro Asn Gln Asp Ser  
 165 170 175  
 Pro Ile Met Glu Gly Lys Thr Pro Ile Leu Gly Leu Asp Val Trp Glu  
 180 185 190  
 His Ala Tyr Tyr Leu Lys Tyr Gln Asn Arg Arg Pro Glu Tyr Ile Ala  
 195 200 205  
 Ala Phe Trp Asn Val Val Asn Trp Asp Glu Val Ala Lys Arg Tyr Ser  
 210 215 220  
 Glu Ala Lys Ala Lys Gln Arg Ser Cys Gly Leu Val Pro Arg Gly Ser  
 225 230 235 240  
 Gly Pro Gly Ser Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp  
 245 250 255

- 11 -

	660							665							670						
5	Ser	Ile	Asn	Gly	Asp	Val	Tyr	Ile	Tyr	Ser	Thr	Asn	Arg	Asn	Gln	Phe					
			675					680					685								
	Gly	Ile	Tyr	Ser	Ser	Lys	Pro	Ser	Glu	Val	Asn	Ile	Ala	Gln	Asn	Asn					
		690					695					700									
10	Asp	Ile	Ile	Tyr	Asn	Gly	Arg	Tyr	Gln	Asn	Phe	Ser	Ile	Ser	Phe	Trp					
	705					710					715					720					
	Val	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Lys	Val	Asn	Leu	Asn	Asn	Glu	Tyr					
					725					730					735						
15	Thr	Ile	Ile	Asp	Cys	Ile	Arg	Asn	Asn	Asn	Ser	Gly	Trp	Lys	Ile	Ser					
				740					745					750							
	Leu	Asn	Tyr	Asn	Lys	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Ala	Gly	Asn					
20			755					760					765								
	Asn	Gln	Lys	Leu	Val	Phe	Asn	Tyr	Thr	Gln	Met	Ile	Ser	Ile	Ser	Asp					
		770					775					780									
25	Tyr	Ile	Asn	Lys	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Gly					
	785					790					795					800					
	Asn	Ser	Arg	Ile	Tyr	Ile	Asn	Gly	Asn	Leu	Ile	Asp	Glu	Lys	Ser	Ile					
					805					810					815						
30	Ser	Asn	Leu	Gly	Asp	Ile	His	Val	Ser	Asp	Asn	Ile	Leu	Phe	Lys	Ile					
				820					825					830							
	Val	Gly	Cys	Asn	Asp	Thr	Arg	Tyr	Val	Gly	Ile	Arg	Tyr	Phe	Lys	Val					
35			835					840					845								
	Phe	Asp	Thr	Glu	Leu	Gly	Lys	Thr	Glu	Ile	Glu	Thr	Leu	Tyr	Ser	Asp					
		850					855					860									
40	Glu	Pro	Asp	Pro	Ser	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asn	Tyr	Leu	Leu					
	865					870					875					880					
	Tyr	Asn	Lys	Arg	Tyr	Tyr	Leu	Leu	Asn	Leu	Leu	Arg	Thr	Asp	Lys	Ser					
					885					890					895						
45	Ile	Thr	Gln	Asn	Ser	Asn	Phe	Leu	Asn	Ile	Asn	Gln	Gln	Arg	Gly	Val					
				900					905					910							
	Tyr	Gln	Lys	Pro	Asn	Ile	Phe	Ser	Asn	Thr	Arg	Leu	Tyr	Thr	Gly	Val					
50			915					920					925								
	Glu	Val	Ile	Ile	Arg	Lys	Asn	Gly	Ser	Thr	Asp	Ile	Ser	Asn	Thr	Asp					
		930					935					940									
55	Asn	Phe	Val	Arg	Lys	Asn	Asp	Leu	Ala	Tyr	Ile	Asn	Val	Val	Asp	Arg					
	945					950					955					960					
	Asp	Val	Glu	Tyr	Arg	Leu	Tyr	Ala	Asp	Ile	Ser	Ile	Ala	Lys	Pro	Glu					
					965					970					975						
60	Lys	Ile	Ile	Lys	Leu	Ile	Arg	Thr	Ser	Asn	Ser	Asn	Asn	Ser	Leu	Gly					
				980					985					990							
	Gln	Ile	Ile	Val	Met	Asp	Ser	Ile	Gly	Asn	Asn	Cys	Thr	Met	Asn	Phe					
65			995				1000						1005								
	Gln	Asn	Asn	Asn	Gly	Gly	Asn	Ile	Gly	Leu	Leu	Gly	Phe	His	Ser	Asn					
		1010					1015					1020									

- 10 -

	Gly	Thr	Ser	Glu	Ile	Glu	Glu	His	Asn	Val	Val	Asp	Leu	Asn	Val	Phe	
	305					310					315					320	
5	Phe	Tyr	Leu	His	Ala	Gln	Lys	Val	Pro	Glu	Gly	Glu	Thr	Asn	Ile	Ser	
					325					330					335		
	Leu	Thr	Ser	Ser	Ile	Asp	Thr	Ala	Leu	Ser	Glu	Glu	Ser	Gln	Val	Tyr	
					340				345					350			
10	Thr	Phe	Phe	Ser	Ser	Glu	Phe	Ile	Asn	Thr	Ile	Asn	Lys	Pro	Val	His	
			355					360					365				
	Ala	Ala	Leu	Phe	Ile	Ser	Trp	Ile	Asn	Gln	Val	Ile	Arg	Asp	Phe	Thr	
15			370				375					380					
	Thr	Glu	Ala	Thr	Gln	Lys	Ser	Thr	Phe	Asp	Lys	Ile	Ala	Asp	Ile	Ser	
	385				390						395					400	
20	Leu	Val	Val	Pro	Tyr	Val	Gly	Leu	Ala	Leu	Asn	Ile	Gly	Asn	Glu	Val	
					405					410					415		
	Gln	Lys	Glu	Asn	Phe	Lys	Glu	Ala	Phe	Glu	Leu	Leu	Gly	Ala	Gly	Ile	
				420					425					430			
25	Leu	Leu	Glu	Phe	Val	Pro	Glu	Leu	Leu	Ile	Pro	Thr	Ile	Leu	Val	Phe	
			435					440					445				
	Thr	Ile	Lys	Ser	Phe	Ile	Gly	Ser	Ser	Glu	Asn	Lys	Asn	Lys	Ile	Ile	
30			450				455					460					
	Lys	Ala	Ile	Asn	Asn	Ser	Leu	Met	Glu	Arg	Glu	Thr	Lys	Trp	Lys	Glu	
	465					470					475					480	
35	Ile	Tyr	Ser	Trp	Ile	Val	Ser	Asn	Trp	Leu	Thr	Arg	Ile	Asn	Thr	Gln	
					485					490					495		
	Phe	Asn	Lys	Arg	Lys	Glu	Gln	Met	Tyr	Glu	Ala	Leu	Gln	Asn	Gln	Val	
				500					505					510			
40	Asp	Ala	Ile	Lys	Thr	Val	Ile	Glu	Tyr	Lys	Tyr	Asn	Asn	Tyr	Thr	Ser	
			515					520					525				
	Asp	Glu	Arg	Asn	Arg	Leu	Glu	Ser	Glu	Tyr	Asn	Ile	Asn	Asn	Ile	Arg	
45			530				535					540					
	Gln	Glu	Leu	Asn	Lys	Lys	Val	Ser	Leu	Ala	Met	Glu	Asn	Ile	Glu	Arg	
	545				550						555					560	
50	Phe	Ile	Thr	Glu	Ser	Ser	Ile	Phe	Tyr	Leu	Met	Lys	Leu	Ile	Asn	Glu	
					565					570					575		
	Ala	Lys	Val	Ser	Lys	Leu	Arg	Glu	Tyr	Asp	Glu	Gly	Val	Lys	Glu	Tyr	
				580					585					590			
55	Leu	Leu	Asp	Tyr	Ile	Ser	Glu	His	Arg	Ser	Ile	Leu	Gly	Asn	Ser	Val	
			595					600					605				
	Gln	Glu	Leu	Asn	Asp	Leu	Val	Thr	Ser	Thr	Leu	Asn	Asn	Ser	Ile	Pro	
60			610				615					620					
	Phe	Glu	Leu	Ser	Ser	Tyr	Thr	Asn	Asp	Lys	Ile	Leu	Ile	Leu	Tyr	Phe	
	625					630					635					640	
65	Asp	Lys	Leu	Tyr	Lys	Lys	Ile	Lys	Asp	Asn	Ser	Ile	Leu	Asp	Met	Arg	
				645					650						655		
	Tyr	Glu	Asn	Asn	Lys	Phe	Ile	Asp	Ile	Ser	Gly	Tyr	Gly	Ser	Asn	Ile	

- 9 -

5  
 <210> 5  
 <211> 1059  
 <212> PRT  
 <213> Artificial Sequence

10  
 <220>  
 <223> Description of Artificial Sequence: construct

15  
 <400> 5  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15

20  
 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30

25  
 Asn Thr Tyr Val Thr Asn Leu Asp Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45

30  
 Leu Gln Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Gln Ala Leu  
 50 55 60

35  
 Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
 65 70 75 80

40  
 Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
 85 90 95

45  
 Pro Thr Gly Glu Leu Ala Asp Ala Ile Asn Lys Lys Phe Gly Ser Phe  
 100 105 110

50  
 Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
 115 120 125

55  
 Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Gln Ile Thr  
 130 135 140

60  
 Ser Thr Pro Asn Gln Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
 145 150 155 160

65  
 Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Gln Asn  
 165 170 175

70  
 Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Val Val Asn Trp Asp  
 180 185 190

75  
 Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys Gln Arg Ser Cys  
 195 200 205

80  
 Gly Leu Val Pro Arg Gly Ser Gly Pro Gly Ser Lys Ala Pro Pro Arg  
 210 215 220

85  
 Leu Cys Ile Arg Val Asn Asn Arg Glu Leu Phe Phe Val Ala Ser Glu  
 225 230 235 240

90  
 Ser Ser Tyr Asn Glu Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp  
 245 250 255

95  
 Thr Thr Asn Leu Asn Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile  
 260 265 270

100  
 Leu Asp Tyr Asn Ser Glu Thr Ile Pro Gln Ile Ser Asn Gln Thr Leu  
 275 280 285

105  
 Asn Thr Leu Val Gln Asp Asp Ser Tyr Val Pro Arg Tyr Asp Ser Asn  
 290 295 300

- 8 -

	705		710		715		720
	Ile Gln Asn Tyr	Ile His Asn Glu Tyr	Thr Ile Ile Asn Cys Met Lys				
		725	730			735	
5	Asn Asn Ser Gly	Trp Lys Ile Ser	Ile Arg Gly Asn Arg Ile Ile Trp				
		740	745			750	
10	Thr Leu Ile Asp	Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr					
		755	760			765	
	Asn Ile Arg Glu Asp	Ile Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val					
		770	775			780	
15	Thr Ile Thr Asn Asn	Leu Asp Asn Ala Lys Ile Tyr Ile Asn Gly Lys					
		790				795	800
	Leu Glu Ser Asn Thr	Asp Ile Lys Asp Ile Arg Glu Val Ile Ala Asn					
		805	810			815	
20	Gly Glu Ile Ile	Phe Lys Leu Asp Gly Asp Ile Asp Arg Thr Gln Phe					
		820	825			830	
25	Ile Trp Met Lys Tyr	Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser					
		835	840			845	
	Asp Ile Glu Glu Arg Tyr	Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys					
		850	855			860	
30	Asp Phe Trp Gly Asn	Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe					
		865	870			875	880
	Asn Ala Gly Asn Lys	Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Pro					
		885	890			895	
35	Val Gly Glu Ile	Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Lys Tyr					
		900	905			910	
40	Ile Asn Tyr Arg Asp	Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg					
		915	920			925	
	Lys Ser Asn Ser Gln Ser	Ile Asn Asp Asp Ile Val Arg Lys Glu Asp					
		930	935			940	
45	Tyr Ile Tyr Leu Asp	Phe Phe Asn Leu Asn Gln Glu Trp Arg Val Tyr					
		945	950			955	960
	Thr Tyr Lys Tyr	Phe Lys Lys Glu Glu Glu Lys Leu Phe Leu Ala Pro					
		965	970			975	
50	Ile Ser Asp Ser Asp	Glu Phe Tyr Asn Thr Ile Gln Ile Lys Glu Tyr					
		980	985			990	
55	Asp Glu Gln Pro Thr Tyr	Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu					
		995	1000			1005	
	Glu Ser Thr Asp Glu	Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu					
		1010	1015			1020	
60	Ser Gly Ile Val Phe	Glu Glu Tyr Lys Asp Tyr Phe Cys Ile Ser Lys					
		1025	1030			1035	1040
	Trp Tyr Leu Lys Glu	Val Lys Arg Lys Pro Tyr Asn Leu Lys Leu Gly					
		1045	1050			1055	
65	Cys Asn Trp Gln Phe	Ile Pro Lys Asp Glu Gly Trp Thr Glu					
		1060	1065			1070	

- 7 -

	Ser	Met	Asp	Tyr	Ile	Lys	Thr	Ala	Asn	Lys	Val	Val	Glu	Ala	Gly	Leu
			355					360					365			
5	Phe	Ala	Gly	Trp	Val	Lys	Gln	Ile	Val	Asn	Asp	Phe	Val	Ile	Glu	Ala
		370					375					380				
	Asn	Lys	Ser	Asn	Thr	Met	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val
		385				390					395				400	
10	Pro	Tyr	Ile	Gly	Leu	Ala	Leu	Asn	Val	Gly	Asn	Glu	Thr	Ala	Lys	Gly
					405					410					415	
	Asn	Phe	Glu	Asn	Ala	Phe	Glu	Ile	Ala	Gly	Ala	Ser	Ile	Leu	Leu	Glu
				420					425					430		
15	Phe	Ile	Pro	Glu	Leu	Leu	Ile	Pro	Val	Val	Gly	Ala	Phe	Leu	Leu	Glu
			435					440					445			
	Ser	Tyr	Ile	Asp	Asn	Lys	Asn	Lys	Ile	Ile	Lys	Thr	Ile	Asp	Asn	Ala
20			450				455					460				
	Leu	Thr	Lys	Arg	Asn	Glu	Lys	Trp	Ser	Asp	Met	Tyr	Gly	Leu	Ile	Val
		465				470					475				480	
25	Ala	Gln	Trp	Leu	Ser	Thr	Val	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	Lys	Glu
					485					490					495	
	Gly	Met	Tyr	Lys	Ala	Leu	Asn	Tyr	Gln	Ala	Gln	Ala	Leu	Glu	Glu	Ile
				500					505					510		
30	Ile	Lys	Tyr	Arg	Tyr	Asn	Ile	Tyr	Ser	Glu	Lys	Glu	Lys	Ser	Asn	Ile
			515					520					525			
	Asn	Ile	Asp	Phe	Asn	Asp	Ile	Asn	Ser	Lys	Leu	Asn	Glu	Gly	Ile	Asn
35			530				535					540				
	Gln	Ala	Ile	Asp	Asn	Ile	Asn	Asn	Phe	Ile	Asn	Gly	Cys	Ser	Val	Ser
		545				550					555				560	
40	Tyr	Leu	Met	Lys	Lys	Met	Ile	Pro	Leu	Ala	Val	Glu	Lys	Leu	Leu	Asp
					565					570					575	
	Phe	Asp	Asn	Thr	Leu	Lys	Lys	Asn	Leu	Leu	Asn	Tyr	Ile	Asp	Glu	Asn
45				580					585					590		
	Lys	Leu	Tyr	Leu	Ile	Gly	Ser	Ala	Glu	Tyr	Glu	Lys	Ser	Lys	Val	Asn
			595					600					605			
	Lys	Tyr	Leu	Lys	Thr	Ile	Met	Pro	Phe	Asp	Leu	Ser	Ile	Tyr	Thr	Asn
50			610				615					620				
	Asp	Thr	Ile	Leu	Ile	Glu	Met	Phe	Asn	Lys	Tyr	Asp	Ser	Glu	Ile	Leu
		625				630					635				640	
55	Asn	Asn	Ile	Ile	Leu	Asn	Leu	Arg	Tyr	Lys	Asp	Asn	Asn	Leu	Ile	Asp
					645					650					655	
	Leu	Ser	Gly	Tyr	Gly	Ala	Lys	Val	Glu	Val	Tyr	Asp	Gly	Val	Glu	Leu
				660					665					670		
60	Asn	Asp	Lys	Asn	Gln	Phe	Lys	Leu	Thr	Ser	Ser	Ala	Asn	Ser	Lys	Ile
			675					680					685			
	Arg	Val	Thr	Gln	Asn	Gln	Asn	Ile	Ile	Phe	Asn	Ser	Val	Phe	Leu	Asp
65			690				695					700				
	Phe	Ser	Val	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Lys	Asn	Asp	Gly



- 6 -

&lt;400&gt; 4

	Met	Pro	Phe	Glu	Leu	Pro	Ala	Leu	Pro	Tyr	Pro	Tyr	Asp	Ala	Leu	Glu
	1				5					10					15	
5	Pro	Sie	Ile	Asp	Lys	Glu	Thr	Met	Asn	Ile	His	His	Thr	Lys	His	His
				20					25					30		
	Asn	Thr	Tyr	Val	Thr	Asn	Leu	Asn	Ala	Ala	Leu	Glu	Gly	His	Pro	Asp
			35					40					45			
10	Leu	Glu	Asn	Lys	Ser	Leu	Glu	Glu	Leu	Leu	Ser	Asn	Leu	Glu	Ala	Leu
			50				55					60				
	Pro	Glu	Ser	Ile	Arg	Thr	Ala	Val	Arg	Asn	Asn	Gly	Gly	Gly	His	Ala
15					70					75						80
	Asn	His	Ser	Leu	Phe	Trp	Thr	Ile	Leu	Ser	Pro	Asn	Gly	Gly	Gly	Glu
					85					90					95	
20	Pro	Thr	Gly	Glu	Leu	Ala	Asp	Ala	Ile	Asn	Lys	Lys	Phe	Gly	Ser	Phe
				100					105					110		
	Thr	Ala	Phe	Lys	Asp	Glu	Phe	Ser	Lys	Ala	Ala	Ala	Gly	Arg	Phe	Gly
25				115				120					125			
	Ser	Gly	Trp	Ala	Trp	Leu	Val	Val	Asn	Asn	Gly	Glu	Leu	Glu	Ile	Thr
			130				135					140				
30	Ser	Thr	Pro	Asn	Gln	Asp	Ser	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile
	145					150					155					160
	Leu	Gly	Leu	Asp	Val	Trp	Glu	His	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn
				165						170					175	
35	Arg	Arg	Pro	Glu	Tyr	Ile	Ala	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp
				180					185					190		
	Glu	Val	Ala	Lys	Arg	Tyr	Ser	Glu	Ala	Lys	Ala	Lys	Glu	Arg	Ser	Cys
			195					200					205			
40	Gly	Leu	Val	Pro	Arg	Gly	Ser	Gly	Pro	Gly	Ser	Lys	Ala	Pro	Gly	Ile
			210				215					220				
	Cys	Ile	Asp	Val	Asp	Asn	Glu	Asp	Leu	Phe	Phe	Ile	Ala	Asp	Lys	Asn
45						220				235						240
	Ser	Phe	Ser	Asp	Asp	Leu	Ser	Lys	Asn	Glu	Arg	Ile	Glu	Tyr	Asn	Thr
				245						250					255	
50	Gln	Ser	Asn	Tyr	Ile	Glu	Asn	Asp	Phe	Pro	Ile	Asn	Glu	Leu	Ile	Leu
				260					265					270		
	Asp	Thr	Asp	Leu	Ile	Ser	Lys	Ile	Glu	Leu	Pro	Ser	Glu	Asn	Thr	Glu
			275					280					285			
55	Ser	Leu	Thr	Asp	Phe	Asn	Val	Asp	Val	Pro	Val	Tyr	Glu	Lys	Gln	Pro
			290				295					300				
	Ala	Ile	Lys	Lys	Ile	Phe	Thr	Asp	Glu	Asn	Thr	Ile	Phe	Gln	Tyr	Leu
60						310					315					320
	Tyr	Ser	Gln	Thr	Phe	Pro	Leu	Asp	Ile	Arg	Asp	Ile	Ser	Leu	Thr	Ser
				325						330					335	
65	Ser	Phe	Asp	Asp	Ala	Leu	Leu	Phe	Ser	Asn	Lys	Val	Tyr	Ser	Phe	Phe
				340					345						350	

- 5 -

Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg  
 770 775 780  
 5 Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asp Asn Ser Lys Ile  
 785 790 795 800  
 Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly  
 805 810 815  
 10 Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg  
 820 825 830  
 Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys  
 835 840 845  
 15 Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn  
 850 855 860  
 20 Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys  
 865 870 875 880  
 Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val  
 885 890 895  
 25 Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly  
 900 905 910  
 30 Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly  
 915 920 925  
 Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile  
 930 935 940  
 35 Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys  
 945 950 955 960  
 Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile  
 965 970 975  
 40 Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val  
 980 985 990  
 Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met  
 995 1000 1005  
 45 Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His  
 1010 1015 1020  
 50 Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg  
 1025 1030 1035 1040  
 Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile  
 1045 1050 1055  
 55 Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu  
 1060 1065  
 60 <210> 4  
 <211> 1070  
 <212> PRT  
 <213> Artificial Sequence  
 65 <220>  
 <223> Description of Artificial Sequence:construct

- 4 -

	405								410								415								
5	Met	Leu	Tyr	Lys 420	Asp	Asp	Phe	Val	Gly 425	Ala	Leu	Ile	Phe	Ser	Gly	Ala									
	Val	Ile	Leu 435	Leu	Glu	Phe	Ile	Pro 440	Glu	Ile	Ala	Ile	Pro 445	Val	Leu	Gly									
10	Thr	Phe 450	Ala	Leu	Val	Ser	Tyr 455	Ile	Ala	Asn	Lys	Val 460	Leu	Thr	Val	Gln									
	Thr 465	Ile	Asp	Asn	Ala	Leu 470	Ser	Lys	Arg	Asn	Glu 475	Lys	Trp	Asp	Glu	Val 480									
15	Tyr	Lys	Tyr	Ile 485	Val	Thr	Asn	Trp	Leu	Ala 490	Lys	Val	Asn	Thr	Gln 495	Ile									
	Asp	Leu	Ile	Arg 500	Lys	Lys	Met	Lys	Glu 505	Ala	Leu	Glu	Asn	Gln 510	Ala	Glu									
20	Ala	Thr	Lys 515	Ala	Ile	Ile	Asn	Tyr 520	Gln	Tyr	Asn	Gln	Tyr 525	Thr	Glu	Glu									
25	Glu	Lys 530	Asn	Asn	Ile	Asn	Phe 535	Asn	Ile	Asp	Asp	Leu 540	Ser	Ser	Lys	Leu									
	Asn 545	Glu	Ser	Ile	Asn	Lys 550	Ala	Met	Ile	Asn 555	Ile	Asn	Lys	Phe	Leu 560	Asn									
30	Gln	Cys	Ser	Val	Ser 565	Tyr	Leu	Met	Asn 570	Ser	Met	Ile	Pro	Tyr	Gly 575	Val									
	Lys	Arg	Leu 580	Glu	Asp	Phe	Asp	Ala	Ser 585	Leu	Lys	Asp	Ala	Leu 590	Leu	Lys									
35	Tyr	Ile	Tyr 595	Asp	Asn	Arg	Gly	Thr 600	Leu	Ile	Gly	Gln	Val 605	Asp	Arg	Leu									
40	Lys	Asp 610	Lys	Val	Asn	Asn	Thr 615	Leu	Ser	Thr	Asp	Ile 620	Pro	Phe	Gln	Leu									
	Ser 625	Lys	Tyr	Val	Asp	Asn 630	Gln	Arg	Leu	Leu	Ser 635	Thr	Phe	Thr	Glu	Tyr 640									
45	Ile	Lys	Asn	Ile 645	Ile	Asn	Thr	Ser	Ile 650	Leu	Asn	Leu	Arg	Tyr	Glu 655	Ser									
	Asn	His	Leu 660	Ile	Asp	Leu	Ser	Arg 665	Tyr	Ala	Ser	Lys	Ile 670	Asn	Ile	Gly									
50	Ser	Lys	Val 675	Asn	Phe	Asp	Pro	Ile 680	Asp	Lys	Asn	Gln 685	Ile	Gln	Leu	Phe									
	Asn 690	Leu	Glu	Ser	Ser	Lys 695	Ile	Glu	Val	Ile	Leu 700	Lys	Asn	Ala	Ile	Val									
55	Tyr 705	Asn	Ser	Met	Tyr	Glu 710	Asn	Phe	Ser	Thr 715	Ser	Phe	Trp	Ile	Arg	Ile 720									
60	Pro	Lys	Tyr	Phe 725	Asn	Ser	Ile	Ser	Leu 730	Asn	Asn	Glu	Tyr	Thr	Ile 735	Ile									
	Asn	Cys	Met	Glu 740	Asn	Asn	Ser	Gly	Trp 745	Lys	Val	Ser	Leu 750	Asn	Tyr	Gly									
65	Glu	Ile 755	Ile	Trp	Thr	Leu	Gln	Asp 760	Thr	Gln	Glu	Ile 765	Lys	Gln	Arg	Val									

- 3 -

	Leu	Gln	Asn	Lys	Ser	Leu	Glu	Glu	Leu	Leu	Ser	Asn	Leu	Glu	Ala	Leu
	50						55					60				
5	Pro	Glu	Ser	Ile	Arg	Thr	Ala	Val	Arg	Asn	Asn	Gly	Gly	Gly	His	Ala
	65					70				75						80
	Asn	His	Ser	Leu	Phe	Trp	Thr	Ile	Leu	Ser	Pro	Asn	Gly	Gly	Gly	Glu
					85					90					95	
10	Pro	Thr	Gly	Glu	Leu	Ala	Asp	Ala	Ile	Asn	Lys	Lys	Phe	Gly	Ser	Phe
				100					105					110		
	Thr	Ala	Phe	Lys	Asp	Glu	Phe	Ser	Lys	Ala	Ala	Ala	Gly	Arg	Phe	Gly
			115					120					125			
15	Ser	Gly	Trp	Ala	Trp	Leu	Val	Val	Asn	Asn	Gly	Glu	Leu	Glu	Ile	Thr
		130					135					140				
	Ser	Thr	Pro	Asn	Glu	Asp	Ser	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile
20		145				150					155					160
	Leu	Gly	Leu	Asp	Val	Trp	Glu	His	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn
				165						170					175	
25	Arg	Arg	Pro	Glu	Tyr	Ile	Ala	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp
				180					185					190		
	Glu	Val	Ala	Lys	Arg	Tyr	Ser	Glu	Ala	Lys	Ala	Lys	Gln	Arg	Ser	Cys
			195					200					205			
30	Gly	Leu	Val	Pro	Arg	Gly	Ser	Gly	Pro	Gly	Ser	Ala	Leu	Asn	Asp	Leu
		210					215					220				
	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp
35		225				230					235					240
	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr
				245					250					255		
40	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln
			260						265					270		
	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile
			275					280					285			
45	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn
		290				295						300				
	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr
50		305				310				315						320
	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg
				325						330				335		
55	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg
				340					345					350		
	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala
			355					360					365			
60	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp
		370					375					380				
	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp
65		385				390					395					400
	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn

- 2 -

5 <210> 2  
 <211> 204  
 <212> PRT  
 <213> *Bacillus stearothermophilus*  
  
 10 <400> 2  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15  
 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30  
 15 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45  
 Leu Glu Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Glu Ala Leu  
 50 55 60  
 20 Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
 65 70 75 80  
 25 Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
 85 90 95  
 Pro Thr Gly Glu Leu Ala Asp Ala Ile Asn Lys Lys Phe Gly Ser Phe  
 100 105 110  
 30 Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
 115 120 125  
 Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Glu Ile Thr  
 130 135 140  
 35 Ser Thr Pro Asn Glu Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
 145 150 155 160  
 40 Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Glu Asn  
 165 170 175  
 Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Val Val Asn Trp Asp  
 180 185 190  
 45 Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys  
 195 200  
  
 50 <210> 3  
 <211> 1067  
 <212> PRT  
 <213> Artificial Sequence  
  
 55 <220>  
 <223> Description of Artificial Sequence: construct  
  
 60 <400> 3  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15  
 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30  
 65 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45

SEQUENCE LISTING

5 <110> Microbiological Research Authority  
HALLIS, Bassam  
SILMAN, Nigel  
SEONE, Clifford Charles  
EUTTON, John Mark

10 <120> Delivery of Superoxide Dismutase to Neuronal Cells  
<130> 20994-SOD-heavy chain conjugates

15 <140>  
<141>

<150> GB 9824282.9  
<151> 1998-11-05

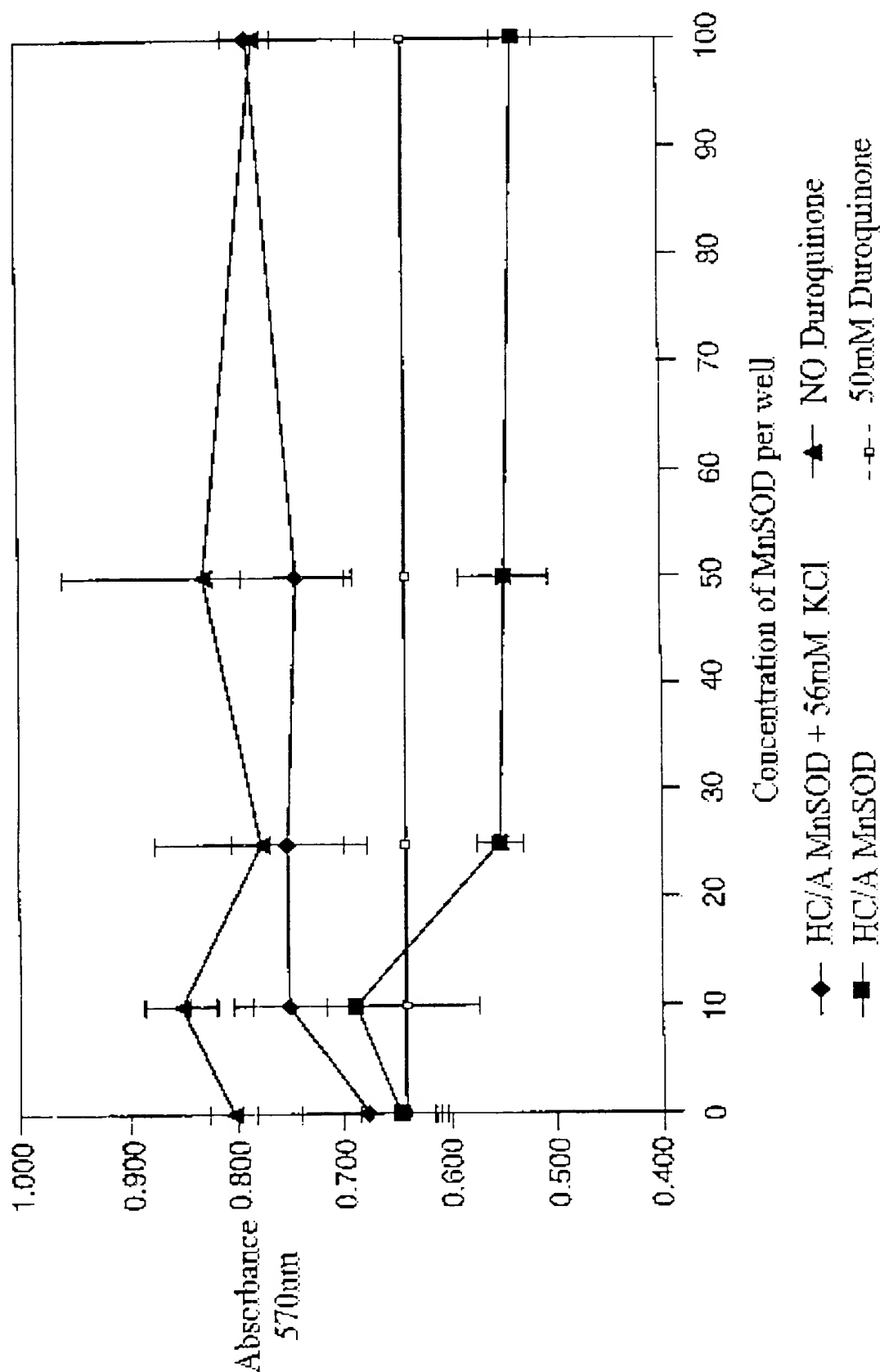
20 <160> 11  
<170> PatentIn Ver. 2.1

25 <210> 1  
<211> 204  
<212> PRT  
<213> Bacillus caldotenax

30 <400> 1  
Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
1 5 10 15  
Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
20 25 30  
35 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
35 40 45  
Leu Glu Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Glu Ala Leu  
50 55 60  
Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
65 70 75 80  
45 Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
85 90 95  
Pro Thr Gly Glu Leu Ala Glu Ala Ile Asn Lys Lys Phe Gly Ser Phe  
100 105 110  
50 Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
115 120 125  
Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Glu Ile Thr  
130 135 140  
55 Ser Thr Pro Asn Glu Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
145 150 155 160  
60 Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Glu Asn  
165 170 175  
Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Ile Val Asp Trp Asp  
180 185 190  
65 Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys  
195 200

4/4

**FIG. 5** Demonstration of protective effects of HC/A MnSOD on NG108 cells subjected to oxidative stress by the addition of 50 $\mu$ M duroquinone for 4 hours.



3/4

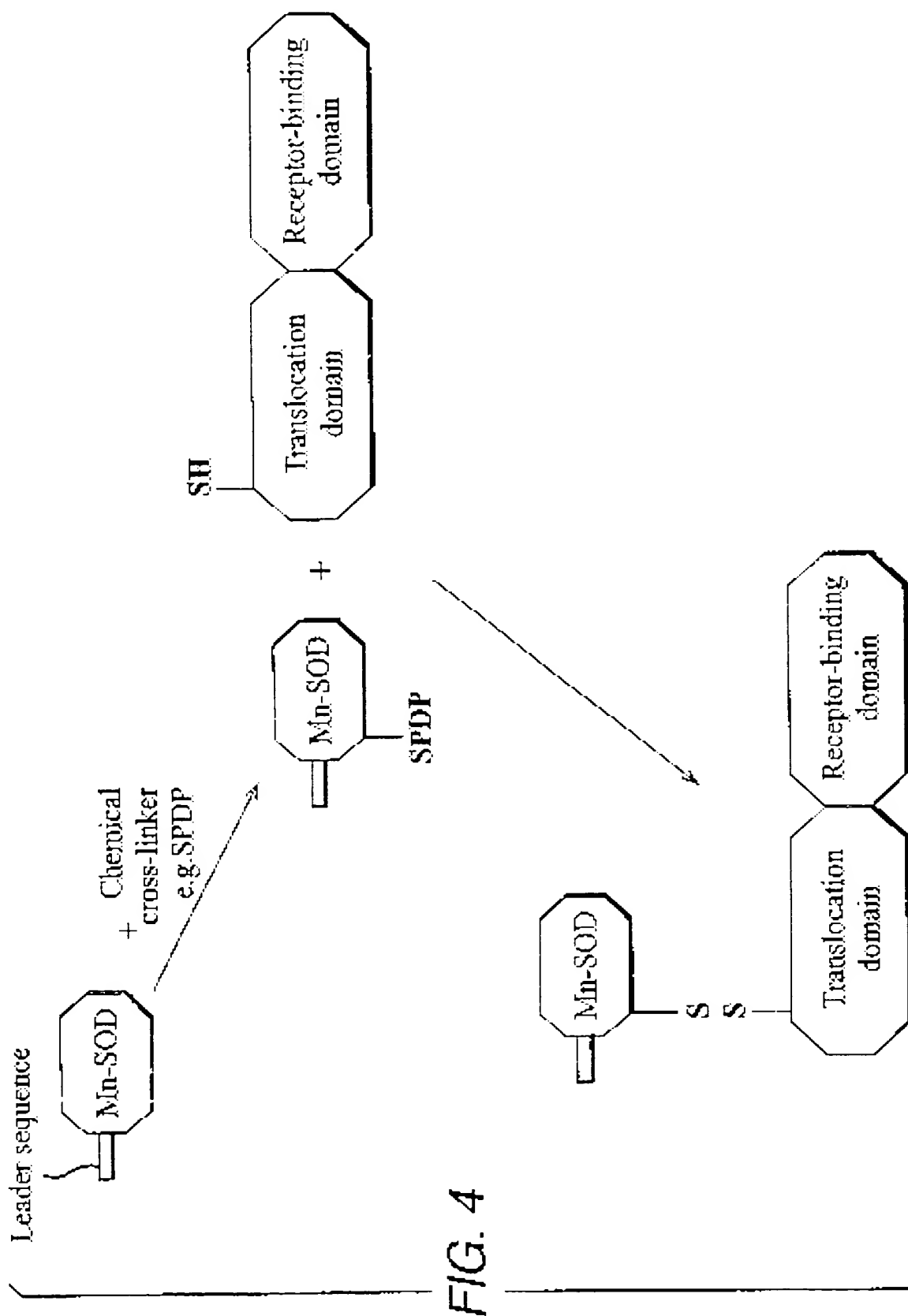
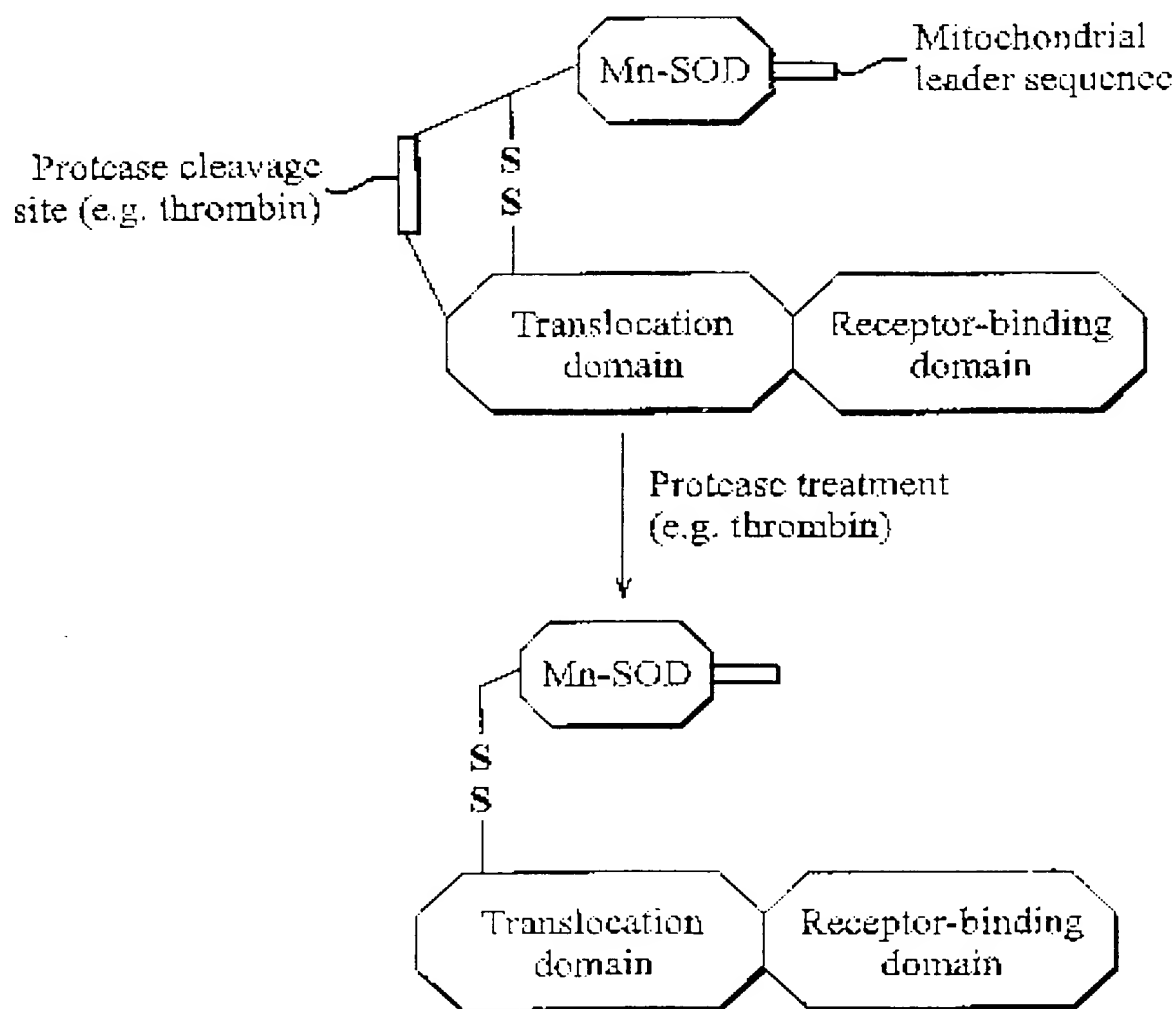


FIG. 4



2/4

FIG. 3



1/4

FIG. 1

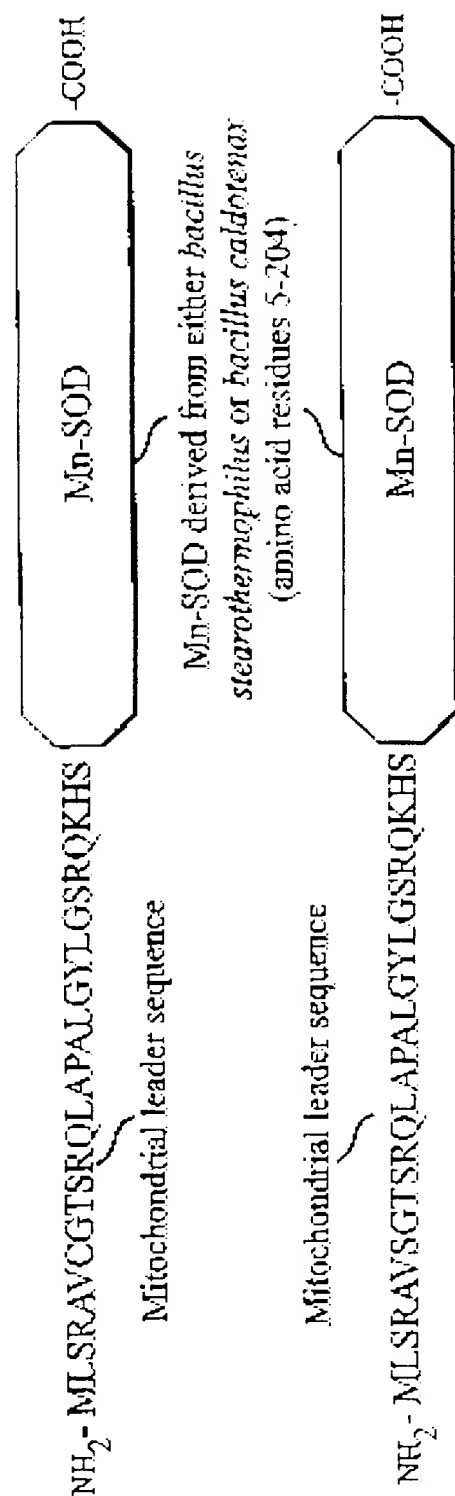
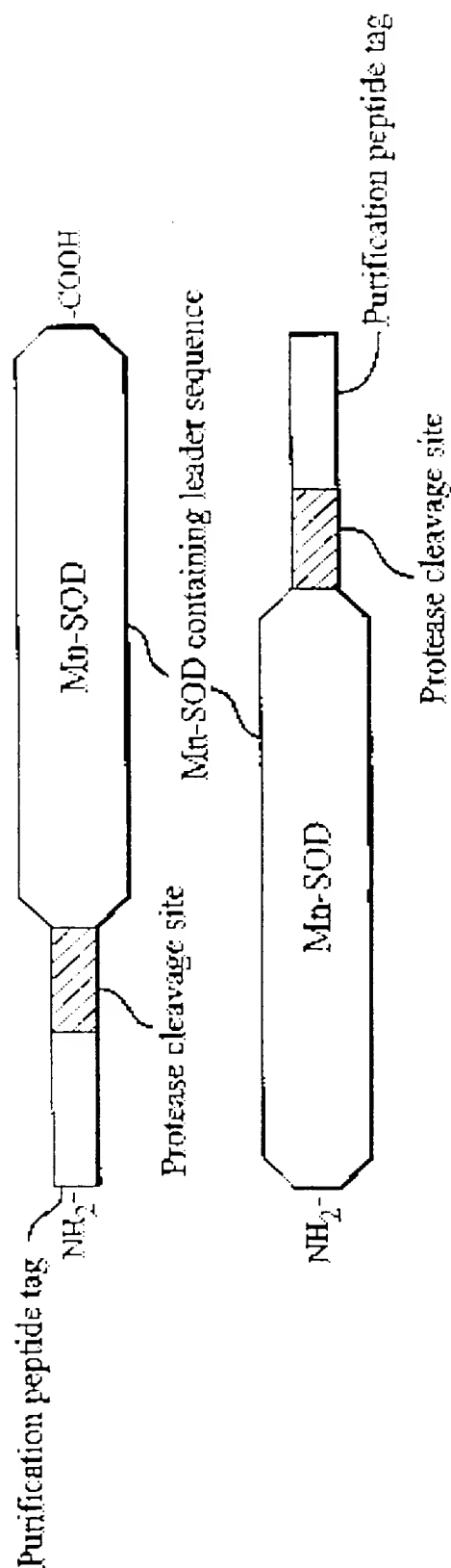


FIG. 2





# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/03699

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5780024	A	14-07-1998	NONE

Form: PCT/ISA/212 (patent family annex) (July 1992)

## INTERNATIONAL SEARCH REPORT

Pat. Application No.

PCT/GB 99/03699

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOWLER C ET AL: "Characterization of the Bacillus stearothermophilus manganese superoxide dismutase gene and its ability to complement copper/zinc superoxide dismutase deficiency in Saccharomyces cerevisiae" JOURNAL OF BACTERIOLOGY, vol. 172, no. 3, 1990, pages 1539-1546, XP000877200 USA abstract	16-22
X	FRANCIS JW ET AL: "CuZn superoxide dismutase (SOD-1): tetanus toxin fragment C hybrid protein for targeted delivery of SOD-1 to neuronal cells" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 25, 1995, pages 15434-15442, XP002131795 MD US abstract; figures 1,4-8	1,6,7,9, 10,13,15
X	US 5 780 024 A (BROWN ROBERT H ET AL) 14 July 1998 (1998-07-14) the whole document	1,6,7,9, 10,13,15

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 99/03699

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/53 C12N15/62 C12N9/02 A61K38/44 A61K48/00  
C07K14/33 A61K39/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FIGUEIREDO D M ET AL: "Delivery of recombinant tetanus -superoxide dismutase proteins to central nervous system neurons by retrograde axonal transport" EXPERIMENTAL NEUROLOGY, US, SAN DIEGO, CA, vol. 145, 1997, pages 546-554, XP002102526 the whole document	1,6,7,9, 10,13,15
Y	FUJII J ET AL: "A defect in the mitochondrial import of mutant Mn-superoxide dismutase produced in Sf21 cells." JOURNAL OF BIOCHEMISTRY, (1998 AUG) 124 (2) 340-6. , XP000867725 page 340 -page 341; figures 1,5 -/-	16-22

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

28 February 2000

Date of mailing of the international search report

15/03/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentean 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Esen, J

- 23 -

&lt;220&gt;

<223> Description of Artificial Sequence: modified human  
mitochondrial leader sequence

5

&lt;400&gt; 11

Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
1 5 10 15

10

Leu Gly Tyr Leu Gly Ser Arg Gln  
20

- 22 -

Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
20 25 30

5 Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
35 40 45

Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
50 55 60

10 Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
65 70 75 80

Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
85 90 95

15 Val Arg Asn Asn Gly Gly Gly His Ala Asn His Ser Leu Phe Trp Thr  
100 105 110

20 Ile Leu Ser Pro Asn Gly Gly Gly Glu Pro Thr Gly Glu Leu Ala Asp  
115 120 125

Ala Ile Asn Lys Lys Phe Gly Ser Phe Thr Ala Phe Lys Asp Glu Phe  
130 135 140

25 Ser Lys Ala Ala Ala Gly Arg Phe Gly Ser Gly Trp Ala Trp Leu Val  
145 150 155 160

Val Asn Asn Gly Glu Leu Glu Ile Thr Ser Thr Pro Asn Gln Asp Ser  
165 170 175

30 Pro Ile Met Glu Gly Lys Thr Pro Ile Leu Gly Leu Asp Val Trp Glu  
180 185 190

35 His Ala Tyr Tyr Leu Lys Tyr Gln Asn Arg Arg Pro Glu Tyr Ile Ala  
195 200 205

Ala Phe Trp Asn Val Val Asn Trp Asp Glu Val Ala Lys Arg Tyr Ser  
210 215 220

40 Glu Ala Lys Ala Lys  
225

45 <210> 10  
<211> 24  
<212> PRT  
<213> Artificial Sequence

50 <220>  
<223> Description of Artificial Sequence: modified human  
mitochondrial leader sequence

<400> 10

55 Met Leu Ser Arg Ala Val Ser Gly Thr Ser Arg Gln Leu Ala Pro Ala  
1 5 10 15

Leu Gly Tyr Leu Gly Ser Arg Gln  
20

60

65 <210> 11  
<211> 24  
<212> PRT  
<213> Artificial Sequence



- 21 -

Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser Arg Ile Tyr Ile Asn  
                     820                    825                    830

5 Gly Asn Leu Ile Asp Glu Lys Ser Ile Ser Asn Leu Gly Asp Ile His  
                     835                    840                    845

Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly Cys Asn Asp Thr Arg  
                     850                    855                    860

10 Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe Asp Thr Glu Leu Gly Lys  
                     865                    870                    875                    880

Thr Glu Ile Glu Thr Leu Tyr Ser Asp Glu Pro Asp Pro Ser Ile Leu  
                     885                    890                    895

15 Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asn Lys Arg Tyr Tyr Leu  
                     900                    905                    910

20 Leu Asn Leu Leu Arg Thr Asp Lys Ser Ile Thr Gln Asn Ser Asn Phe  
                     915                    920                    925

Leu Asn Ile Asn Gln Gln Arg Gly Val Tyr Gln Lys Pro Asn Ile Phe  
                     930                    935                    940

25 Ser Asn Thr Arg Leu Tyr Thr Gly Val Glu Val Ile Ile Arg Lys Asn  
                     945                    950                    955                    960

Gly Ser Thr Asp Ile Ser Asn Thr Asp Asn Phe Val Arg Lys Asn Asp  
                     965                    970                    975

30 Leu Ala Tyr Ile Asn Val Val Asp Arg Asp Val Glu Tyr Arg Leu Tyr  
                     980                    985                    990

35 Ala Asp Ile Ser Ile Ala Lys Pro Glu Lys Ile Ile Lys Leu Ile Arg  
                     995                    1000                    1005

Thr Ser Asn Ser Asn Asn Ser Leu Gly Gln Ile Ile Val Met Asp Ser  
                     1010                    1015                    1020

40 Ile Gly Asn Asn Cys Thr Met Asn Phe Gln Asn Asn Asn Gly Gly Asn  
                     1025                    1030                    1035                    1040

Ile Gly Leu Leu Gly Phe His Ser Asn Asn Leu Val Ala Ser Ser Trp  
                     1045                    1050                    1055

45 Tyr Tyr Asn Asn Ile Arg Lys Asn Thr Ser Ser Asn Gly Cys Phe Trp  
                     1060                    1065                    1070

50 Ser Phe Ile Ser Lys Glu His Gly Trp Gln Glu Asn  
                     1075                    1080

55 <210> 9  
       <211> 229  
       <212> PRT  
       <213> Artificial Sequence

60 <220>  
       <223> Description of Artificial Sequence: polypeptide  
               comprising a mitochondrial leader from human MnSOD  
               and B. Stearothermophilus SOD

65 <400> 9  
       Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
           1                    5                    10                    15

- 20 -

	450					455					460					
5	Leu 465	Leu	Ile	Pro	Thr	Ile 470	Leu	Val	Phe	Thr	Ile 475	Lys	Ser	Phe	Ile	Gly 480
	Ser	Ser	Glu	Asn	Lys 485	Asn	Lys	Ile	Ile	Lys 490	Ala	Ile	Asn	Asn	Ser	Leu
10	Met	Glu	Arg	Glu 500	Thr	Lys	Trp	Lys	Glu 505	Ile	Tyr	Ser	Trp	Ile 510	Val	Ser
	Asn	Trp	Leu 515	Thr	Arg	Ile	Asn	Thr 520	Gln	Phe	Asn	Lys	Arg 525	Lys	Glu	Gln
15	Met	Tyr 530	Gln	Ala	Leu	Gln	Asn 535	Gln	Val	Asp	Ala	Ile 540	Lys	Thr	Val	Ile
20	Glu 545	Tyr	Lys	Tyr	Asn	Asn 550	Tyr	Thr	Ser	Asp	Glu 555	Arg	Asn	Arg	Leu	Glu 560
	Ser	Glu	Tyr	Asn	Ile 565	Asn	Asn	Ile	Arg	Glu 570	Glu	Leu	Asn	Lys	Lys 575	Val
25	Ser	Leu	Ala	Met 580	Glu	Asn	Ile	Glu	Arg 585	Phe	Ile	Thr	Glu	Ser 590	Ser	Ile
	Phe	Tyr	Leu 595	Met	Lys	Leu	Ile	Asn 600	Glu	Ala	Lys	Val	Ser 605	Lys	Leu	Arg
30	Glu	Tyr 610	Asp	Glu	Gly	Val	Lys 615	Glu	Tyr	Leu	Leu	Asp 620	Tyr	Ile	Ser	Glu
35	His 625	Arg	Ser	Ile	Leu	Gly 630	Asn	Ser	Val	Gln	Glu 635	Leu	Asn	Asp	Leu	Val 640
	Thr	Ser	Thr	Leu	Asn 645	Asn	Ser	Ile	Pro	Phe 650	Glu	Leu	Ser	Ser	Tyr 655	Thr
40	Asn	Asp	Lys	Ile 660	Leu	Ile	Leu	Tyr	Phe 665	Asn	Lys	Leu	Tyr	Lys 670	Lys	Ile
	Lys	Asp	Asn 675	Ser	Ile	Leu	Asp 680	Met	Arg	Tyr	Glu	Asn 685	Asn	Lys	Phe	Ile
45	Asp	Ile 690	Ser	Gly	Tyr	Gly 695	Ser	Asn	Ile	Ser	Ile 700	Asn	Gly	Asp	Val	Tyr
50	Ile 705	Tyr	Ser	Thr	Asn 710	Arg	Asn	Gln	Phe	Gly	Ile 715	Tyr	Ser	Ser	Lys	Pro 720
	Ser	Glu	Val	Asn 725	Ile	Ala	Gln	Asn	Asn	Asp 730	Ile	Ile	Tyr	Asn 735	Gly	Arg
55	Tyr	Gln	Asn 740	Phe	Ser	Ile	Ser	Phe 745	Trp	Val	Arg	Ile	Pro 750	Lys	Tyr	Phe
	Asn	Lys 755	Val	Asn	Leu	Asn	Asn 760	Glu	Tyr	Thr	Ile	Ile 765	Asp	Cys	Ile	Arg
60	Asn 770	Asn	Asn	Ser	Gly	Trp 775	Lys	Ile	Ser	Leu	Asn 780	Tyr	Asn	Lys	Ile	Ile
65	Trp 785	Thr	Leu	Gln	Asp 790	Thr	Ala	Gly	Asn	Asn	Gln 795	Lys	Leu	Val	Phe	Asn 800
	Tyr	Thr	Gln	Met 805	Ile	Ser	Ile	Ser	Asp 810	Tyr	Ile	Asn	Lys	Trp 815	Ile	Phe

- 19 -

	Val	Arg	Asn	Asn	Gly	Gly	Gly	His	Ala	Asn	His	Ser	Leu	Phe	Trp	Thr	
				100					105					110			
5	Ile	Leu	Ser	Pro	Asn	Gly	Gly	Gly	Glu	Pro	Thr	Gly	Glu	Leu	Ala	Asp	
			115					120					125				
	Ala	Ile	Asn	Lys	Lys	Phe	Gly	Ser	Phe	Thr	Ala	Phe	Lys	Asp	Glu	Phe	
		130					135					140					
10	Ser	Lys	Ala	Ala	Ala	Gly	Arg	Phe	Gly	Ser	Gly	Trp	Ala	Trp	Leu	Val	
	145					150					155					160	
	Val	Asn	Asn	Gly	Glu	Leu	Glu	Ile	Thr	Ser	Thr	Pro	Asn	Gln	Asp	Ser	
				165						170					175		
15	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile	Leu	Gly	Leu	Asp	Val	Trp	Glu	
				180					185					190			
20	His	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn	Arg	Arg	Pro	Glu	Tyr	Ile	Ala	
			195					200					205				
	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp	Glu	Val	Ala	Lys	Arg	Tyr	Ser	
		210					215					220					
25	Glu	Ala	Lys	Ala	Lys	Gln	Arg	Ser	Cys	Gly	Leu	Val	Pro	Arg	Gly	Ser	
	225					230					235					240	
	Gly	Pro	Gly	Ser	Lys	Ala	Pro	Pro	Arg	Leu	Cys	Ile	Arg	Val	Asn	Asn	
					245					250					255		
30	Arg	Glu	Leu	Phe	Phe	Val	Ala	Ser	Glu	Ser	Ser	Tyr	Asn	Glu	Asn	Asp	
				260					265					270			
35	Ile	Asn	Thr	Pro	Lys	Glu	Ile	Asp	Asp	Thr	Thr	Asn	Leu	Asn	Asn	Asn	
			275					280					285				
	Tyr	Arg	Asn	Asn	Leu	Asp	Glu	Val	Ile	Leu	Asp	Tyr	Asn	Ser	Glu	Thr	
		290				295						300					
40	Ile	Pro	Gln	Ile	Ser	Asn	Gln	Thr	Leu	Asn	Thr	Leu	Val	Gln	Asp	Asp	
	305					310					315					320	
	Ser	Tyr	Val	Pro	Arg	Tyr	Asp	Ser	Asn	Gly	Thr	Ser	Glu	Ile	Glu	Glu	
					325					330					335		
45	His	Asn	Val	Val	Asp	Leu	Asn	Val	Phe	Phe	Tyr	Leu	His	Ala	Gln	Lys	
				340					345					350			
50	Val	Pro	Glu	Gly	Glu	Thr	Asn	Ile	Ser	Leu	Thr	Ser	Ser	Ile	Asp	Thr	
			355				360						365				
	Ala	Leu	Ser	Glu	Glu	Ser	Gln	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Glu	Phe	
		370					375					380					
55	Ile	Asn	Thr	Ile	Asn	Lys	Pro	Val	His	Ala	Ala	Leu	Phe	Ile	Ser	Trp	
	385					390					395					400	
	Ile	Asn	Gln	Val	Ile	Arg	Asp	Phe	Thr	Thr	Glu	Ala	Thr	Gln	Lys	Ser	
				405						410					415		
60	Thr	Phe	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Val	Val	Pro	Tyr	Val	Gly	
				420					425					430			
65	Leu	Ala	Leu	Asn	Ile	Gly	Asn	Glu	Val	Gln	Lys	Glu	Asn	Phe	Lys	Glu	
		435					440						445				
	Ala	Phe	Glu	Leu	Leu	Gly	Ala	Gly	Ile	Leu	Leu	Glu	Phe	Val	Pro	Glu	

- 18 -

Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly Asn Lys Asn Ser  
                     900                    905                    910

5 Tyr Ile Lys Leu Lys Lys Asp Ser Pro Val Gly Glu Ile Leu Thr Arg  
                     915                    920                    925

Ser Lys Tyr Asn Gln Asn Ser Lys Tyr Ile Asn Tyr Arg Asp Leu Tyr  
                     930                    935                    940

10 Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn Ser Gln Ser Ile  
                     945                    950                    955                    960

Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr Leu Asp Phe Phe  
                     965                    970                    975

15 Asn Leu Asn Gln Glu Trp Arg Val Tyr Thr Tyr Lys Tyr Phe Lys Lys  
                     980                    985                    990

Glu Glu Glu Lys Leu Phe Leu Ala Pro Ile Ser Asp Ser Asp Glu Phe  
                     995                    1000                    1005

20 Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln Pro Thr Tyr Ser  
                     1010                    1015                    1020

25 Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr Asp Glu Ile Gly  
                     1025                    1030                    1035                    1040

Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile Val Phe Glu Glu  
                     1045                    1050                    1055

30 Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu Lys Glu Val Lys  
                     1060                    1065                    1070

35 Arg Lys Pro Tyr Asn Leu Lys Leu Gly Cys Asn Trp Gln Phe Ile Pro  
                     1075                    1080                    1085

Lys Asp Glu Gly Trp Thr Glu  
                     1090                    1095

40

<210> 8  
 <211> 1084  
 <212> PRT  
 <213> Artificial Sequence

45

<220>  
 <223> Description of Artificial Sequence:construct

50

<400> 8  
 Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
   1                    5                    10                    15

55 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
                     20                    25                    30

Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
                     35                    40                    45

60 Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
                     50                    55                    60

Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
                     65                    70                    75                    80

65 Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
                     85                    90                    95

- 17 -

	530		535			540													
5	Tyr 545	Ser	Glu	Lys	Glu 550	Lys	Ser	Asn	Ile	Asn 555	Ile	Asp	Phe	Asn	Asp	Ile 560			
	Asn	Ser	Lys	Leu	Asn 565	Glu	Gly	Ile	Asn	Gln 570	Ala	Ile	Asp	Asn	Ile 575	Asn			
10	Asn	Phe	Ile	Asn 580	Gly	Cys	Ser	Val	Ser 585	Tyr	Leu	Met	Lys	Lys 590	Met	Ile			
	Pro	Leu	Ala 595	Val	Glu	Lys	Leu	Leu 600	Asp	Phe	Asp	Asn	Thr 605	Leu	Lys	Lys			
15	Asn 610	Leu	Leu	Asn	Tyr	Ile	Asp 615	Glu	Asn	Lys	Leu	Tyr 620	Leu	Ile	Gly	Ser			
	Ala 625	Glu	Tyr	Glu	Lys 630	Ser	Lys	Val	Asn	Lys	Tyr 635	Leu	Lys	Thr	Ile	Met 640			
20	Pro	Phe	Asp	Leu	Ser 645	Ile	Tyr	Thr	Asn	Asp 650	Thr	Ile	Leu	Ile	Glu 655	Met			
	Phe	Asn	Lys	Tyr 660	Asn	Ser	Glu	Ile	Leu 665	Asn	Asn	Ile	Ile	Leu 670	Asn	Leu			
25	Arg	Tyr	Lys 675	Asp	Asn	Asn	Leu	Ile 680	Asp	Leu	Ser	Gly	Tyr 685	Gly	Ala	Lys			
30	Val 690	Glu	Val	Tyr	Asp	Gly	Val 695	Glu	Leu	Asn	Asp	Lys 700	Asn	Gln	Phe	Lys			
	Leu 705	Thr	Ser	Ser	Ala	Asn 710	Ser	Lys	Ile	Arg	Val 715	Thr	Gln	Asn	Gln	Asn 720			
35	Ile	Ile	Phe	Asn	Ser 725	Val	Phe	Leu	Asp	Phe 730	Ser	Val	Ser	Phe	Trp 735	Ile			
40	Arg	Ile	Pro	Lys 740	Tyr	Lys	Asn	Asp	Gly 745	Ile	Gln	Asn	Tyr	Ile 750	His	Asn			
	Glu	Tyr	Thr 755	Ile	Ile	Asn	Cys	Met 760	Lys	Asn	Asn	Ser	Gly 765	Trp	Lys	Ile			
45	Ser 770	Ile	Arg	Gly	Asn	Arg	Ile 775	Ile	Trp	Thr	Leu	Ile 780	Asp	Ile	Asn	Gly			
	Lys 785	Thr	Lys	Ser	Val 790	Phe	Phe	Glu	Tyr	Asn 795	Ile	Arg	Glu	Asp	Ile	Ser 800			
50	Glu	Tyr	Ile	Asn	Arg 805	Trp	Phe	Phe	Val	Thr 810	Ile	Thr	Asn	Asn	Leu 815	Asn			
55	Asn	Ala	Lys	Ile 820	Tyr	Ile	Asn	Gly	Lys 825	Leu	Glu	Ser	Asn	Thr 830	Asp	Ile			
	Lys	Asp	Ile 835	Arg	Glu	Val	Ile	Ala 840	Asn	Gly	Glu	Ile	Ile	Phe 845	Lys	Leu			
60	Asp 850	Gly	Asp	Ile	Asp	Arg	Thr 855	Gln	Phe	Ile	Trp	Met 860	Lys	Tyr	Phe	Ser			
	Ile 865	Phe	Asn	Thr	Glu	Leu 870	Ser	Gln	Ser	Asn	Ile 875	Glu	Glu	Arg	Tyr	Lys 880			
65	Ile	Gln	Ser	Tyr	Ser 885	Glu	Tyr	Leu	Lys	Asp 890	Phe	Trp	Gly	Asn	Pro 895	Leu			

- 16 -

	Pro	Ile	Met	Glu	Gly	Lys	Thr	Pro	Ile	Leu	Gly	Leu	Asp	Val	Trp	Glu	
				180					185					190			
5	His	Ala	Tyr	Tyr	Leu	Lys	Tyr	Gln	Asn	Arg	Arg	Pro	Glu	Tyr	Ile	Ala	
			195					200					205				
	Ala	Phe	Trp	Asn	Val	Val	Asn	Trp	Asp	Glu	Val	Ala	Lys	Arg	Tyr	Ser	
		210					215					220					
10	Glu	Ala	Lys	Ala	Lys	Gln	Arg	Ser	Cys	Gly	Leu	Val	Pro	Arg	Gly	Ser	
	225					230					235					240	
	Gly	Pro	Gly	Ser	Lys	Ala	Pro	Gly	Ile	Cys	Ile	Asp	Val	Asp	Asn	Glu	
					245					250					255		
15	Asp	Leu	Phe	Phe	Ile	Ala	Asp	Lys	Asn	Ser	Phe	Ser	Asp	Asp	Leu	Ser	
			260					265						270			
20	Lys	Asn	Glu	Arg	Ile	Glu	Tyr	Asn	Thr	Gln	Ser	Asn	Tyr	Ile	Glu	Asn	
			275					280					285				
	Asp	Phe	Pro	Ile	Asn	Glu	Leu	Ile	Leu	Asp	Thr	Asp	Leu	Ile	Ser	Lys	
		290					295					300					
25	Ile	Glu	Leu	Pro	Ser	Glu	Asn	Thr	Glu	Ser	Leu	Thr	Asp	Phe	Asn	Val	
	305					310					315					320	
	Asp	Val	Pro	Val	Tyr	Glu	Lys	Gln	Pro	Ala	Ile	Lys	Lys	Ile	Phe	Thr	
					325					330					335		
30	Asp	Glu	Asn	Thr	Ile	Phe	Gln	Tyr	Leu	Tyr	Ser	Gln	Thr	Phe	Pro	Leu	
				340					345					350			
35	Asp	Ile	Arg	Asp	Ile	Ser	Leu	Thr	Ser	Ser	Phe	Asp	Asp	Ala	Leu	Leu	
			355					360					365				
	Phe	Ser	Asn	Lys	Val	Tyr	Ser	Phe	Phe	Ser	Met	Asp	Tyr	Ile	Lys	Thr	
		370					375					380					
40	Ala	Asn	Lys	Val	Val	Glu	Ala	Gly	Leu	Phe	Ala	Gly	Trp	Val	Lys	Gln	
	385					390					395					400	
	Ile	Val	Asn	Asp	Phe	Val	Ile	Glu	Ala	Asn	Lys	Ser	Asn	Thr	Met	Asp	
					405					410					415		
45	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val	Pro	Tyr	Ile	Gly	Leu	Ala	Leu	
				420					425					430			
50	Asn	Val	Gly	Asn	Glu	Thr	Ala	Lys	Gly	Asn	Phe	Glu	Asn	Ala	Phe	Glu	
			435					440					445				
	Ile	Ala	Gly	Ala	Ser	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Leu	Leu	Ile	
		450					455					460					
55	Pro	Val	Val	Gly	Ala	Phe	Leu	Leu	Glu	Ser	Tyr	Ile	Asp	Asn	Lys	Asn	
	465					470					475					480	
	Lys	Ile	Ile	Lys	Thr	Ile	Asp	Asn	Ala	Leu	Thr	Lys	Arg	Asn	Glu	Lys	
					485					490					495		
60	Trp	Ser	Asp	Met	Tyr	Gly	Leu	Ile	Val	Ala	Gln	Trp	Leu	Ser	Thr	Val	
				500					505					510			
65	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	Lys	Glu	Gly	Met	Tyr	Lys	Ala	Leu	Asn	
		515						520					525				
	Tyr	Gln	Ala	Gln	Ala	Leu	Glu	Glu	Ile	Ile	Lys	Tyr	Arg	Tyr	Asn	Ile	

- 15 -

Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn  
                     980                    985                    990

5 Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro  
                     995                    1000                    1005

Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp  
                     1010                    1015                    1020

10 Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly  
                     1025                    1030                    1035                    1040

Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys  
                     1045                    1050                    1055

15 Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg  
                     1060                    1065                    1070

20 Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly  
                     1075                    1080                    1085

Glu Arg Pro Leu  
                     1090

25

<210> 7  
 <211> 1095  
 <212> PRT  
 30 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:construct

35 <400> 7  
 Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
                     1                    5                    10                    15

40 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
                     20                    25                    30

Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
                     35                    40                    45

45 Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
                     50                    55                    60

Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
                     65                    70                    75                    80

50 Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
                     85                    90                    95

55 Val Arg Asn Asn Gly Gly Gly His Ala Asn His Ser Leu Phe Trp Thr  
                     100                    105                    110

Ile Leu Ser Pro Asn Gly Gly Gly Glu Pro Thr Gly Glu Leu Ala Asp  
                     115                    120                    125

60 Ala Ile Asn Lys Lys Phe Gly Ser Phe Thr Ala Phe Lys Asp Glu Phe  
                     130                    135                    140

Ser Lys Ala Ala Ala Gly Arg Phe Gly Ser Gly Trp Ala Trp Leu Val  
                     145                    150                    155                    160

65 Val Asn Asn Gly Glu Leu Glu Ile Thr Ser Thr Pro Asn Gln Asp Ser  
                     165                    170                    175

- 14 -

	610	615	620
5	Thr Leu Ile Gly Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr 625 630 635 640		
	Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln 645 650 655		
10	Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr 660 665 670		
	Ser Ile Leu Asn Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser 675 680 685		
15	Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro 690 695 700		
	Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile 705 710 715 720		
20	Glu Val Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn 725 730 735		
25	Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile 740 745 750		
	Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser 755 760 765		
30	Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln 770 775 780		
	Asp Thr Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met 785 790 795 800		
35	Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr 805 810 815		
40	Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile 820 825 830		
	Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn 835 840 845		
45	Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp 850 855 860		
	Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile 865 870 875 880		
50	Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe 885 890 895		
55	Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu 900 905 910		
	Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly 915 920 925		
60	Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile 930 935 940		
	Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys 945 950 955 960		
65	Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val 965 970 975		



- 13 -

	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn
				260					265					270		
5	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu
			275					280					285			
	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe
		290					295					300				
10	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile
	305					310					315					320
	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly
					325					330					335	
15	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala
				340					345					350		
20	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val
			355					360					365			
	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser
		370					375					380				
25	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu
	385					390					395					400
	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu
					405					410					415	
30	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr
				420					425					430		
35	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe
			435					440					445			
	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile
		450					455					460				
40	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr
	465					470					475					480
	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser
					485					490					495	
45	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn
				500					505					510		
50	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met
			515					520					525			
	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn
		530					535					540				
55	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe
	545					550					555					560
	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala
					565					570					575	
60	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu
				580					585					590		
	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp
				595				600					605			
65	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly

- 12 -

Asn Leu Val Ala Ser Ser Trp Tyr Tyr Asn Asn Ile Arg Lys Asn Thr  
1025 1030 1035 1040

5 Ser Ser Asn Gly Cys Phe Trp Ser Phe Ile Ser Lys Glu His Gly Trp  
1045 1050 1055

Gln Glu Asn

10

<210> 6  
<211> 1092  
<212> PRT  
15 <213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:construct

20 <400> 6  
Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala  
1 5 10 15

25 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Arg Gly Ser Pro Ala  
20 25 30

Leu Pro Tyr Pro Tyr Asp Ala Leu Glu Pro His Ile Asp Lys Glu Thr  
35 40 45

30 Met Asn Ile His His Thr Lys His His Asn Thr Tyr Val Thr Asn Leu  
50 55 60

35 Asn Ala Ala Leu Glu Gly His Pro Asp Leu Gln Asn Lys Ser Leu Glu  
65 70 75 80

Glu Leu Leu Ser Asn Leu Glu Ala Leu Pro Glu Ser Ile Arg Thr Ala  
85 90 95

40 Val Arg Asn Asn Gly Gly Gly His Ala Asn His Ser Leu Phe Trp Thr  
100 105 110

Ile Leu Ser Pro Asn Gly Gly Gly Glu Pro Thr Gly Glu Leu Ala Asp  
115 120 125

45 Ala Ile Asn Lys Lys Phe Gly Ser Phe Thr Ala Phe Lys Asp Glu Phe  
130 135 140

50 Ser Lys Ala Ala Ala Gly Arg Phe Gly Ser Gly Trp Ala Trp Leu Val  
145 150 155 160

Val Asn Asn Gly Glu Leu Glu Ile Thr Ser Thr Pro Asn Gln Asp Ser  
165 170 175

55 Pro Ile Met Glu Gly Lys Thr Pro Ile Leu Gly Leu Asp Val Trp Glu  
180 185 190

His Ala Tyr Tyr Leu Lys Tyr Gln Asn Arg Arg Pro Glu Tyr Ile Ala  
195 200 205

60 Ala Phe Trp Asn Val Val Asn Trp Asp Glu Val Ala Lys Arg Tyr Ser  
210 215 220

Glu Ala Lys Ala Lys Gln Arg Ser Cys Gly Leu Val Pro Arg Gly Ser  
225 230 235 240

65 Gly Pro Gly Ser Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp  
245 250 255

- 11 -

	660	665	670
5	Ser Ile Asn Gly Asp Val Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe 675 680 685		
	Gly Ile Tyr Ser Ser Lys Pro Ser Glu Val Asn Ile Ala Gln Asn Asn 690 695 700		
10	Asp Ile Ile Tyr Asn Gly Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp 705 710 715 720		
	Val Arg Ile Pro Lys Tyr Phe Asn Lys Val Asn Leu Asn Asn Glu Tyr 725 730 735		
15	Thr Ile Ile Asp Cys Ile Arg Asn Asn Asn Ser Gly Trp Lys Ile Ser 740 745 750		
	Leu Asn Tyr Asn Lys Ile Ile Trp Thr Leu Gln Asp Thr Ala Gly Asn 755 760 765		
20	Asn Gln Lys Leu Val Phe Asn Tyr Thr Gln Met Ile Ser Ile Ser Asp 770 775 780		
	Tyr Ile Asn Lys Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Gly 785 790 795 800		
25	Asn Ser Arg Ile Tyr Ile Asn Gly Asn Leu Ile Asp Glu Lys Ser Ile 805 810 815		
30	Ser Asn Leu Gly Asp Ile His Val Ser Asp Asn Ile Leu Phe Lys Ile 820 825 830		
	Val Gly Cys Asn Asp Thr Arg Tyr Val Gly Ile Arg Tyr Phe Lys Val 835 840 845		
35	Phe Asp Thr Glu Leu Gly Lys Thr Glu Ile Glu Thr Leu Tyr Ser Asp 850 855 860		
	Glu Pro Asp Pro Ser Ile Leu Lys Asp Phe Trp Gly Asn Tyr Leu Leu 865 870 875 880		
40	Tyr Asn Lys Arg Tyr Tyr Leu Leu Asn Leu Leu Arg Thr Asp Lys Ser 885 890 895		
45	Ile Thr Gln Asn Ser Asn Phe Leu Asn Ile Asn Gln Gln Arg Gly Val 900 905 910		
	Tyr Gln Lys Pro Asn Ile Phe Ser Asn Thr Arg Leu Tyr Thr Gly Val 915 920 925		
50	Glu Val Ile Ile Arg Lys Asn Gly Ser Thr Asp Ile Ser Asn Thr Asp 930 935 940		
	Asn Phe Val Arg Lys Asn Asp Leu Ala Tyr Ile Asn Val Val Asp Arg 945 950 955 960		
55	Asp Val Glu Tyr Arg Leu Tyr Ala Asp Ile Ser Ile Ala Lys Pro Glu 965 970 975		
60	Lys Ile Ile Lys Leu Ile Arg Thr Ser Asn Ser Asn Asn Ser Leu Gly 980 985 990		
	Gln Ile Ile Val Met Asp Ser Ile Gly Asn Asn Cys Thr Met Asn Phe 995 1000 1005		
65	Gln Asn Asn Asn Gly Gly Asn Ile Gly Leu Leu Gly Phe His Ser Asn 1010 1015 1020		

- 10 -

	Gly	Thr	Ser	Glu	Ile	Glu	Glu	His	Asn	Val	Val	Asp	Leu	Asn	Val	Phe	
	305					310					315					320	
5	Phe	Tyr	Leu	His	Ala	Gln	Lys	Val	Pro	Glu	Gly	Glu	Thr	Asn	Ile	Ser	
					325					330					335		
	Leu	Thr	Ser	Ser	Ile	Asp	Thr	Ala	Leu	Ser	Glu	Glu	Ser	Gln	Val	Tyr	
				340					345					350			
10	Thr	Phe	Phe	Ser	Ser	Glu	Phe	Ile	Asn	Thr	Ile	Asn	Lys	Pro	Val	His	
			355					360					365				
	Ala	Ala	Leu	Phe	Ile	Ser	Trp	Ile	Asn	Gln	Val	Ile	Arg	Asp	Phe	Thr	
		370					375					380					
15	Thr	Glu	Ala	Thr	Gln	Lys	Ser	Thr	Phe	Asp	Lys	Ile	Ala	Asp	Ile	Ser	
	385					390					395					400	
	Leu	Val	Val	Pro	Tyr	Val	Gly	Leu	Ala	Leu	Asn	Ile	Gly	Asn	Glu	Val	
20					405					410					415		
	Gln	Lys	Glu	Asn	Phe	Lys	Glu	Ala	Phe	Glu	Leu	Leu	Gly	Ala	Gly	Ile	
				420					425					430			
25	Leu	Leu	Glu	Phe	Val	Pro	Glu	Leu	Leu	Ile	Pro	Thr	Ile	Leu	Val	Phe	
			435					440					445				
	Thr	Ile	Lys	Ser	Phe	Ile	Gly	Ser	Ser	Glu	Asn	Lys	Asn	Lys	Ile	Ile	
30		450					455					460					
	Lys	Ala	Ile	Asn	Asn	Ser	Leu	Met	Glu	Arg	Glu	Thr	Lys	Trp	Lys	Glu	
	465					470				475						480	
	Ile	Tyr	Ser	Trp	Ile	Val	Ser	Asn	Trp	Leu	Thr	Arg	Ile	Asn	Thr	Gln	
35					485					490					495		
	Phe	Asn	Lys	Arg	Lys	Glu	Gln	Met	Tyr	Gln	Ala	Leu	Gln	Asn	Gln	Val	
				500					505					510			
40	Asp	Ala	Ile	Lys	Thr	Val	Ile	Glu	Tyr	Lys	Tyr	Asn	Asn	Tyr	Thr	Ser	
			515					520					525				
	Asp	Glu	Arg	Asn	Arg	Leu	Glu	Ser	Glu	Tyr	Asn	Ile	Asn	Asn	Ile	Arg	
45		530					535					540					
	Glu	Glu	Leu	Asn	Lys	Lys	Val	Ser	Leu	Ala	Met	Glu	Asn	Ile	Glu	Arg	
	545					550					555					560	
	Phe	Ile	Thr	Glu	Ser	Ser	Ile	Phe	Tyr	Leu	Met	Lys	Leu	Ile	Asn	Glu	
50					565				570						575		
	Ala	Lys	Val	Ser	Lys	Leu	Arg	Glu	Tyr	Asp	Glu	Gly	Val	Lys	Glu	Tyr	
				580					585					590			
55	Leu	Leu	Asp	Tyr	Ile	Ser	Glu	His	Arg	Ser	Ile	Leu	Gly	Asn	Ser	Val	
			595					600					605				
	Gln	Glu	Leu	Asn	Asp	Leu	Val	Thr	Ser	Thr	Leu	Asn	Asn	Ser	Ile	Pro	
60		610					615					620					
	Phe	Glu	Leu	Ser	Ser	Tyr	Thr	Asn	Asp	Lys	Ile	Leu	Ile	Leu	Tyr	Phe	
	625					630					635					640	
	Asn	Lys	Leu	Tyr	Lys	Lys	Ile	Lys	Asp	Asn	Ser	Ile	Leu	Asp	Met	Arg	
65					645					650					655		
	Tyr	Glu	Asn	Asn	Lys	Phe	Ile	Asp	Ile	Ser	Gly	Tyr	Gly	Ser	Asn	Ile	

- 9 -

5 <210> 5  
 <211> 1059  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 10 <223> Description of Artificial Sequence:construct  
  
 <400> 5  
 Met Pro Phe Glu Leu Pro Ala Leu Pro Tyr Pro Tyr Asp Ala Leu Glu  
 1 5 10 15  
 15 Pro His Ile Asp Lys Glu Thr Met Asn Ile His His Thr Lys His His  
 20 25 30  
 Asn Thr Tyr Val Thr Asn Leu Asn Ala Ala Leu Glu Gly His Pro Asp  
 35 40 45  
 20 Leu Gln Asn Lys Ser Leu Glu Glu Leu Leu Ser Asn Leu Glu Ala Leu  
 50 55 60  
 25 Pro Glu Ser Ile Arg Thr Ala Val Arg Asn Asn Gly Gly Gly His Ala  
 65 70 75 80  
 Asn His Ser Leu Phe Trp Thr Ile Leu Ser Pro Asn Gly Gly Gly Glu  
 85 90 95  
 30 Pro Thr Gly Glu Leu Ala Asp Ala Ile Asn Lys Lys Phe Gly Ser Phe  
 100 105 110  
 Thr Ala Phe Lys Asp Glu Phe Ser Lys Ala Ala Ala Gly Arg Phe Gly  
 115 120 125  
 35 Ser Gly Trp Ala Trp Leu Val Val Asn Asn Gly Glu Leu Glu Ile Thr  
 130 135 140  
 40 Ser Thr Pro Asn Gln Asp Ser Pro Ile Met Glu Gly Lys Thr Pro Ile  
 145 150 155 160  
 Leu Gly Leu Asp Val Trp Glu His Ala Tyr Tyr Leu Lys Tyr Gln Asn  
 165 170 175  
 45 Arg Arg Pro Glu Tyr Ile Ala Ala Phe Trp Asn Val Val Asn Trp Asp  
 180 185 190  
 Glu Val Ala Lys Arg Tyr Ser Glu Ala Lys Ala Lys Gln Arg Ser Cys  
 195 200 205  
 Gly Leu Val Pro Arg Gly Ser Gly Pro Gly Ser Lys Ala Pro Pro Arg  
 210 215 220  
 Leu Cys Ile Arg Val Asn Asn Arg Glu Leu Phe Phe Val Ala Ser Glu  
 225 230 235 240  
 Ser Ser Tyr Asn Glu Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp  
 245 250 255  
 Thr Thr Asn Leu Asn Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile  
 260 265 270  
 Leu Asp Tyr Asn Ser Glu Thr Ile Pro Gln Ile Ser Asn Gln Thr Leu  
 275 280 285  
 sn Thr Leu Val Gln Asp Asp Ser Tyr Val Pro Arg Tyr Asp Ser Asn  
 290 295 300

- 8 -

	705		710		715		720									
	Ile	Gln	Asn	Tyr	Ile	His	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Lys
					725					730					735	
5	Asn	Asn	Ser	Gly	Trp	Lys	Ile	Ser	Ile	Arg	Gly	Asn	Arg	Ile	Ile	Trp
				740					745					750		
10	Thr	Leu	Ile	Asp	Ile	Asn	Gly	Lys	Thr	Lys	Ser	Val	Phe	Phe	Glu	Tyr
			755					760					765			
	Asn	Ile	Arg	Glu	Asp	Ile	Ser	Glu	Tyr	Ile	Asn	Arg	Trp	Phe	Phe	Val
		770					775					780				
15	Thr	Ile	Thr	Asn	Asn	Leu	Asn	Asn	Ala	Lys	Ile	Tyr	Ile	Asn	Gly	Lys
	785					790					795					800
	Leu	Glu	Ser	Asn	Thr	Asp	Ile	Lys	Asp	Ile	Arg	Glu	Val	Ile	Ala	Asn
					805					810					815	
20	Gly	Glu	Ile	Ile	Phe	Lys	Leu	Asp	Gly	Asp	Ile	Asp	Arg	Thr	Gln	Phe
				820					825					830		
	Ile	Trp	Met	Lys	Tyr	Phe	Ser	Ile	Phe	Asn	Thr	Glu	Leu	Ser	Gln	Ser
25			835					840					845			
	Asn	Ile	Glu	Glu	Arg	Tyr	Lys	Ile	Gln	Ser	Tyr	Ser	Glu	Tyr	Leu	Lys
		850					855					860				
30	Asp	Phe	Trp	Gly	Asn	Pro	Leu	Met	Tyr	Asn	Lys	Glu	Tyr	Tyr	Met	Phe
	865					870					875					880
	Asn	Ala	Gly	Asn	Lys	Asn	Ser	Tyr	Ile	Lys	Leu	Lys	Lys	Asp	Ser	Pro
				885						890					895	
35	Val	Gly	Glu	Ile	Leu	Thr	Arg	Ser	Lys	Tyr	Asn	Gln	Asn	Ser	Lys	Tyr
				900					905					910		
	Ile	Asn	Tyr	Arg	Asp	Leu	Tyr	Ile	Gly	Glu	Lys	Phe	Ile	Ile	Arg	Arg
40			915					920					925			
	Lys	Ser	Asn	Ser	Gln	Ser	Ile	Asn	Asp	Asp	Ile	Val	Arg	Lys	Glu	Asp
		930					935					940				
45	Tyr	Ile	Tyr	Leu	Asp	Phe	Phe	Asn	Leu	Asn	Gln	Glu	Trp	Arg	Val	Tyr
	945				950					955						960
	Thr	Tyr	Lys	Tyr	Phe	Lys	Lys	Glu	Glu	Glu	Lys	Leu	Phe	Leu	Ala	Pro
				965					970						975	
50	Ile	Ser	Asp	Ser	Asp	Glu	Phe	Tyr	Asn	Thr	Ile	Gln	Ile	Lys	Glu	Tyr
				980					985					990		
	Asp	Glu	Gln	Pro	Thr	Tyr	Ser	Cys	Gln	Leu	Leu	Phe	Lys	Lys	Asp	Glu
55			995				1000					1005				
	Glu	Ser	Thr	Asp	Glu	Ile	Gly	Leu	Ile	Gly	Ile	His	Arg	Phe	Tyr	Glu
		1010					1015					1020				
60	Ser	Gly	Ile	Val	Phe	Glu	Glu	Tyr	Lys	Asp	Tyr	Phe	Cys	Ile	Ser	Lys
	1025				1030					1035						1040
	Trp	Tyr	Leu	Lys	Glu	Val	Lys	Arg	Lys	Pro	Tyr	Asn	Leu	Lys	Leu	Gly
				1045					1050						1055	
65	Cys	Asn	Trp	Gln	Phe	Ile	Pro	Lys	Asp	Glu	Gly	Trp	Thr	Glu		
			1060					1065					1070			